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(54) **NOVEL OSTEOINDUCTIVE COMPOSITIONS**

**OSTEOINDUKTIVE MITTEL**

**NOUVELLES COMPOSITIONS OSTEOINDUCTIVES**

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## Description

The present invention relates to novel proteins, processes for obtaining them and genes encoding them. These proteins are capable of inducing cartilage and bone formation.

### Background

Bone is a highly specialized tissue characterized by an extensive matrix structure formed of fibrous bundles of the protein collagen, and proteoglycans, noncollagenous proteins, lipids and acidic proteins. The processes of bone formation and renewal/repair of bone tissue, which occur continuously throughout life, are performed by specialized cells. Normal embryonic long bone development is preceded by formation of a cartilage model. Bone growth is presumably mediated by "osteoblasts" (bone-forming cells), while remodeling of bone is apparently accomplished by the joint activities of bone-resorbing cells, called "osteoclasts" and osteoblasts. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

### Brief Description of the Invention

The present invention provides novel proteins in purified form and genes encoding them. Specifically, two of the novel proteins are designated BMP-2 Class I (or BMP-2), and BMP-2 Class II (or BMP-4) wherein BMP is bone morphogenic protein. These proteins are characterized by peptide sequences the same as or substantially homologous to amino acid sequences illustrated in Tables II, III and IV below. They are capable of inducing bone formation at a predetermined site. These bone inductive factors are further characterized by biochemical and biological characteristics including activity at a concentration of 10 to 1000ng/gram of bone in an *in vivo* rat bone formation assay described below. Proteins of this invention may be encoded by the DNA sequences depicted in the Tables or by sequences capable of hybridizing thereto and coding for polypeptides with bone growth factor biological properties or other variously modified sequences demonstrating such properties.

One of the proteins of the invention is designated BMP-2 Class I (or BMP-2). It is characterized by at least a portion of a peptide sequence the same or substantially the same as that of amino acid #1 through amino acid #396 of Table III which represents the cDNA hBMP-2 Class I. This peptide sequence is encoded by the same or substantially the same DNA sequence, as depicted in nucleotide #356 through nucleotide #1543 of Table III. The human peptide sequence identified in Table III is 396 amino acids in length. hBMP-2 or related bone inductive proteins may also be characterized by at least a portion of this peptide sequence. hBMP-2 Class I is further characterized by the ability to induce bone formation.

The homologous bovine bone inductive protein of the invention designated bBMP-2 Class I (or bBMP-2), has a DNA sequence identified in Table II below which represents the genomic sequence. This bovine DNA sequence has a prospective 129 amino acid coding sequence followed by approximately 205 nucleotides (a presumptive 3' non-coding sequence). bBMP-2, Class I is further characterized by the ability to induce bone formation. A further bone inductive protein composition of the invention is designated BMP-2 Class II or BMP-4. The human protein hBMP-2 Class II (or hBMP-4) is characterized by at least a portion of the same or substantially the same peptide sequence between amino acid #1 through amino acid #408 of Table IV, which represents the cDNA of hBMP-2 Class II. This peptide sequence is encoded by at least a portion of the same or substantially the same DNA sequence as depicted in nucleotide #403 through nucleotide #1626 of Table IV. This factor is further characterized by the ability to induce bone formation.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of one or more bone growth factor polypeptides according to the invention in a pharmaceutically acceptable vehicle. These compositions may further include other therapeutically useful agents. They may also include an appropriate matrix for delivering the proteins to the site of the bone defect and for providing a structure for bone growth. These compositions may be employed in methods for treating a number of bone defects and periodontal disease. These methods, according to the invention, entail administering to a patient needing such bone formation an effective amount of at least one of the novel proteins BMP-2 Class I and BMP-2 Class-II as described herein.

Still a further aspect of the invention are DNA sequences coding on expression for a human or bovine polypeptide having the ability to induce bone formation. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Tables II, III and IV. Alternatively, a DNA sequence which hybridizes under stringent conditions with the DNA sequences of Tables II, III and IV or a DNA sequence which hybridizes under non-stringent conditions with the illustrated DNA sequences and which codes on expression for a protein having at least one bone growth factor biological property are included in the present invention. Finally, allelic or other variations of the sequences of Tables II, III and IV, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present

invention.

Still a further aspect of the invention is a vector containing a DNA sequence as described above in operative association with an expression control sequence. Such vector may be employed in a novel process for producing a bone growth factor polypeptide in which a cell line transformed with a DNA sequence encoding expression of a bone growth factor polypeptide in operative association with an expression control sequence therefor, is cultured. This claimed process may employ a number of known cells as host cells for expression of the polypeptide. Presently preferred cell lines are mammalian cell lines and bacterial cells.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

#### Detailed Description of the Invention

The proteins of the present invention are characterized by amino acid sequences or portions thereof the same as or substantially homologous to the sequences shown in Tables II, III and IV. These proteins are also characterized by the ability to induce bone formation.

The bone growth factors provided herein also include factors encoded by the sequences similar to those of Tables II, III and IV, but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Tables II, III and IV. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Tables II, III and IV may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring bone growth factor polypeptides in therapeutic processes.

Other specific mutations of the sequences of the bone growth factors described herein involve modifications of one or both of the glycosylation sites. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one or both of the asparagine-linked glycosylation recognition sites present in the sequences of the bone growth factors shown in Tables II, III and IV. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for bone growth factors. These DNA sequences include those depicted in Tables II, III and IV in a 5' to 3' direction and those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences of Tables II, III and IV.

DNA sequences which hybridize to the sequences of Tables II, III and IV under relaxed hybridization conditions and which code on expression for bone growth factors having bone growth factor biological properties also encode bone growth factors of the invention. For example, a DNA sequence which shares regions of significant homology, e.g., sites of glycosylation or disulfide linkages, with the sequences of Tables II, III and IV and encodes a bone growth factor having one or more bone growth factor biological properties clearly encodes a member of this novel family of growth factors, even if such a DNA sequence would not stringently hybridize to the sequence of Tables II, III and IV.

Similarly, DNA sequences which code for bone growth factor polypeptides coded for by the sequences of Tables II, III and IV, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel growth factors described herein. Variations in the DNA sequences of Tables II, III and IV which are caused by point mutations or by induced modifications to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the novel osteoinductive factors. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a DNA sequence coding on expression for a novel bone growth factor polypeptide of the invention, under the control of known regulatory sequences. Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary (CHO) cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293: 620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Bacterial cells are suitable hosts. For example, the various strains of *E. coli* (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Pseudomonas*, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, Genetic Engineering, 8:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel osteoinductive polypeptides. Preferably the vectors contain the full novel DNA sequences described above which code for the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the bone inductive protein sequences. Alternatively, vectors incorporating modified sequences as described above are also embodiments of the present invention and useful in the production of the bone inductive proteins. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does not form part of the present invention.

A protein of the present invention, which induces bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures. An osteogenic preparation employing one or more of the proteins of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. An osteogenic factor of the invention may be valuable in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Of course, the proteins of the invention may have other therapeutic uses.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to bone defects or periodontal diseases. Such a composition comprises a therapeutically effective amount of at least one of the bone inductive factor proteins of the invention. The bone inductive factors according to the present invention may be present in a therapeutic composition in admixture with a pharmaceutically acceptable vehicle or matrix. Further therapeutic methods and compositions of the invention comprise a therapeutic amount of a bone inductive factor of the invention with a therapeutic amount of at least one of the other bone inductive factors of the invention. Additionally, the proteins according to the present invention or a combination of the proteins of the present invention may be co-administered with one or more different osteoinductive factors with which they may interact. Further, the bone inductive proteins may be combined with other agents beneficial to the treatment of the bone defect in question. Such agents include, but are not limited to various growth factors. The preparation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

In particular, BMP-2 Class I may be used individually in a pharmaceutical composition. BMP-2 Class I may also be used in combination with one or more of the other proteins of the invention. BMP-2 Class I may be combined with BMP-2 Class II. It may also be combined with BMP-3. Further BMP-2 Class I may be combined with BMP-2 Class II and BMP-3.

BMP-2 Class II may be used individually in pharmaceutical composition. In addition, it may be used in combination with other proteins as identified above. Further it may be used in combination with BMP-3.

The therapeutic method includes locally administering the composition as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone damage. Preferably, the bone growth inductive factor composition would include a matrix capable of delivering the bone inductive factor to the site of bone damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of other materials presently in use for other implanted medical applications.

The choice of material is based on, for example, biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. Similarly, the application of the osteoinductive factors will define the appropriate formulation. Potential matrices for the osteoinductive factors may be biodegradable and chemically defined, such as, but not limited to calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyanhydrides; biodegradable and biologically well defined, such as bone or dermal collagen, other pure proteins or extracellular matrix components; nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics; or combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics might also be altered in composition, such as in calcium-aluminate-phos-

phate and processing to alter for example, pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of such a growth factor, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the composition of BMP's. The addition of other known growth factors, such as IGF 1 (insulin like growth factor 1), to the final composition, may also effect the dosage. Generally, the dosage regimen should be in the range of approximately 10 to 10<sup>6</sup> nanograms of protein per gram of bone weight desired. Progress can be monitored by periodic assessment of bone growth and/or repair, e.g. x-rays. Such therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in bone inductive factors. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the bone inductive factors of the present invention.

The following examples illustrate practice of the present invention in recovering and characterizing the bovine proteins and employing them to recover the human proteins, obtaining the human proteins and in expressing the proteins via recombinant techniques.

#### EXAMPLE I

##### Isolation of Bovine Bone Inductive Factor

Ground bovine bone powder (20-120 mesh, Helitrex) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in successive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 16 hours at 4°C with 50 liters of 2M CaCl<sub>2</sub> and 10mM ethylenediamine-tetraacetic acid [EDTA], and followed by extraction for 4 hours in 50 liters of 0.5M EDTA. The residue is washed three times with distilled water before its resuspension in 20 liters of 4M guanidine hydrochloride [GuCl], 20mM Tris (pH 7.4), 1 mM N-ethylmaleimide, 1mM iodoacetamide, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 10 liters of GuCl buffer. The residue is extracted for another 24 hours.

The crude GuCl extracts are combined, concentrated approximately 20 times on a Pellicon apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 50mM Tris, 0.1M NaCl, 6M urea (pH7.2), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 4 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the bone inductive factor containing material desorbed from the column by 50mM NaAc, 0.25mM NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40- fold, then diluted 5 times with 80mM KPO<sub>4</sub>, 6M urea (pH6.0). The pH of the solution is adjusted to 6.0 with 500mM K<sub>2</sub>HPO<sub>4</sub>. The sample is applied to an hydroxylapatite column (LKB) equilibrated in 80mM KPO<sub>4</sub>, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Bone inductive factor activity is eluted with 100mM KPO<sub>4</sub> (pH7.4) and 6M urea.

The protein is concentrated approximately 10 times, and solid NaCl added to a final concentration of 0.15M. This material is applied to a heparin - Sepharose column equilibrated in 50mM KPO<sub>4</sub>, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone inductive factor activity is eluted by 50mM KPO<sub>4</sub>, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated to a minimum volume, and 0.4ml aliquots are applied to Superose 6 and Superose 12 columns connected in series, equilibrated with 4M GuCl, 20mM Tris (pH7.2) and the columns developed at a flow rate of 0.25ml/min. The protein demonstrating bone inductive factor activity has a relative migration corresponding to approximately 30,000 dalton protein.

The above fractions are pooled, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). Active fractions are pooled and brought to pH3.0 with 10% trifluoroacetic acid (TFA). The material is applied to a 0.46 x 25cm Vydac C4 column in 0.1% TFA and the column developed with a gradient to 90% acetonitrile, 0.1% TFA (31.5% acetonitrile, 0.1% TFA to 49.5% acetonitrile, 0.1% TFA in 60 minutes at 1ml per minute). Active material is eluted at approximately 40-44% acetonitrile. Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 257:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis and urea Triton X 100 isoelectric focusing. At this stage, the bone inductive factor is estimated to be approximately 10-50% pure.

## EXAMPLE II

Characterization of Bovine Bone Inductive Factor

## A. Molecular Weight

Approximately 20ug protein from Example I is lyophilized and redissolved in 1X SDS sample buffer. After 15 minutes of heating at 37°C, the sample is applied to a 15% SDS polyacrylamide gel and then electrophoresed with cooling. The molecular weight is determined relative to prestained molecular weight standards (Bethesda Research Labs). Immediately after completion, the gel lane containing bone inductive factor is sliced into 0.3cm pieces. Each piece is mashed and 1.4ml of 0.1% SDS is added. The samples are shaken gently overnight at room temperature to elute the protein. Each gel slice is desalted to prevent interference in the biological assay. The supernatant from each sample is acidified to pH 3.0 with 10% TFA, filtered through a 0.45 micron membrane and loaded on a 0.46cm x 5cm C4 Vydac column developed with a gradient of 0.1% TFA to 0.1% TFA, 90% CH<sub>3</sub>CN. The appropriate bone inductive factor - containing fractions are pooled and reconstituted with 20mg rat matrix. In this gel system, the majority of bone inductive factor fractions have the mobility of a protein having a molecular weight of approximately 28,000 - 30,000 daltons.

## B. Isoelectric Focusing

The isoelectric point of bone inductive factor activity is determined in a denaturing isoelectric focusing system. The Triton X100 urea gel system (Hoeffer Scientific) is modified as follows: 1) 40% of the ampholytes used are Servalyte 3/10; 60% are Servalyte 7-9. 2) The catholyte used is 40mM NaOH. Approximately 20ug of protein from Example I is lyophilized, dissolved in sample buffer and applied to the isoelectrofocusing gel. The gel is run at 20 watts, 10°C for approximately 3 hours. At completion the lane containing bone inductive factor is sliced into 0.5 cm slices. Each piece is mashed in 1.0ml 6M urea, 5mM Tris (pH 7.8) and the samples agitated at room temperature. The samples are acidified, filtered, desalted and assayed as described above. The major portion of activity as determined in the assay described in Example III migrates in a manner consistent with a pI of 8.8 - 9.2.

## C. Subunit Characterization

The subunit composition of bone inductive factor is also determined. Pure bone inductive factor is isolated from a preparative 15% SDS gel as described above. A portion of the sample is then reduced with 5mM DTT in sample buffer and re-electrophoresed on a 15% SDS gel. The approximately 30kd protein yields two major bands at approximately 20kd and 18kd, as well as a minor band at 30kd. The broadness of the two bands indicates heterogeneity caused most probably by glycosylation, other post translational modification, proteolytic degradation or carbamylation.

## EXAMPLE III

Biological Activity of Bone Inductive Factor

A rat bone formation assay according to the general procedure of Sampath and Reddi, Proc. Natl. Acad. Sci. U. S.A., 80:6591-6595 (1983) is used to evaluate the osteogenic activity of the bovine bone inductive factor of the present invention obtained in Example I. This assay can also be used to evaluate bone inductive factors of other species. The ethanol precipitation step is replaced by dialyzing the fraction to be assayed against water. The solution or suspension is then redissolved in a volatile solvent, e.g. 0.1 - 0.2 % TFA, and the resulting solution added to 20mg of rat matrix. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., Proc. Natl. Acad. Sci., 69:1601 (1972)] and half is fixed and processed for histological analysis. Routinely, 1µm glycolmethacrylate sections are stained with Von Kossa and acid fuchsin to detect new bone mineral. Alkaline phosphatase, an enzyme produced by chondroblasts and osteoblasts in the process of matrix formation, is also measured. New cartilage and bone formation often correlates with alkaline phosphatase levels. Table I below illustrates the dose response of the rat matrix samples including a control not treated with bone inductive factor.

TABLE 1

Protein* Implanted $\mu$ g	Cartilage	Alk. Phos. u/l
7.5	2	Not done
2.5	3	445.7
0.83	3	77.4
0.28	0	32.5
0.00	0	31.0

\*At this stage the bone inductive factor is approximately 10-15% pure.

The bone or cartilage formed is physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing as described above, followed by autoradiography. Analysis reveals a correlation of activity with protein bands at 28 - 30kd and a pI 9.0. An extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and approximating the purity of bone inductive factor in a particular fraction. In the in vivo rat bone formation assays on dilutions as described above, the protein is active in vivo at 10 to 200ng protein/gram bone to probably greater than 1  $\mu$ g protein/gram bone.

#### EXAMPLE IV

##### Bovine Bone Inductive Factor Protein Composition

The protein composition of Example IIA of molecular weight 28 - 30kd is reduced as described in Example IIC and digested with trypsin. Eight tryptic fragments are isolated by standard procedures having the following amino acid sequences:

Fragment 1: A A F L G D I A L D E E D L G

Fragment 2: A F Q V Q Q A A D L

Fragment 3: N Y Q D M V V E G

Fragment 4: S T P A Q D V S R

Fragment 5: N Q E A L R

Fragment 6: L S E P D P S H T L E E

Fragment 7: F D A Y Y

Fragment 8: L K P S N ? A T I Q S I V E

A less highly purified preparation of protein from bovine bone is prepared according to a purification scheme similar to that described in Example I. The purification basically varies from that previously described by omission of the DE-52 column, the CM cellulose column and the mono s column, as well as a reversal in the order of the hydroxylapatite and heparin sepharose columns. Briefly, the concentrated crude 4 M extract is brought to 85% final concentration of ethanol at 4 degrees. The mixture is then centrifuged, and the precipitate redissolved in 50 mM Tris, 0.15 M NaCl, 6.0 M urea. This material is then fractionated on Heparin Sepharose as described. The Heparin bound material is fractionated on hydroxyapatite as described. The active fractions are pooled, concentrated, and fractionated on a high resolution gel filtration (TSK 30000 in 6 M guanidinium chloride, 50 mM Tris, pH 7.2). The active fractions are pooled, dialyzed against 0.1% TFA, and then fractionated on a C4 Vydac reverse phase column as described. The preparation is reduced and electrophoresed on an acrylamide gel. The protein corresponding to the 18K band is eluted and digested with trypsin. Tryptic fragments are isolated having the following amino acid sequences:

Fragment 9: S L K P S N H A T I Q S ? V

Fragment 10: S F D A Y Y C S ? A

Fragment 11: V Y P N M T V E S C A

Fragment 12: V D F A D I ? W

Tryptic Fragments 7 and 8 are noted to be substantially the same as Fragments 10 and 9, respectively.

A. bBMF-2

Two probes consisting of pools of oligonucleotides are designed on the basis of the amino acid sequence of Fragment 3 and synthesized on an automated DNA synthesizer as described above.

**Probe #1: A C N A C C A T [A/G] T C [T/C] T G [A/G] A T**

**Probe #2: C A [A/G] G A [T/C] A T G G T N G T N G A**

These probes are radioactively labeled and employed to screen the bovine genomic library constructed as follows: Bovine liver DNA is partially digested with the restriction endonuclease enzyme Sau 3A and sedimented through a sucrose gradient. Size fractionated DNA in the range of 15-30kb is then ligated to the lambda J1 BamH1 arms vector [Frischauf et al, *J. Mol. Biol.*, 170:827-842 (1983) Mullins et al., *Nature* 308: 856-858 (1984)]. The library is plated at 8000 recombinants per plate. Duplicate nitrocellulose replicas of the plaques are made and amplified according to a modification of the procedure of Woo et al, *Proc. Natl. Acad. Sci. USA*, 75:3688-91 (1978).

The radioactively labelled 17-mer Probe #1 is hybridized to the set of filters according to the following method:

The probe is kinased and hybridized to the other set of filters in 3M tetramethylammonium chloride (TMAC), 0.1M sodium phosphate pH6.5, 1mM EDTA, 5X Denhardts, 0.6% SDS, 100ug/ml salmon sperm DNA at 48 degrees C, and washed in 3M TMAC, 50mM Tris pH8.0 at 50 degrees C. These conditions minimize the detection of mismatches to the probe pool [see, Wood et al, *Proc. Natl. Acad. Sci. U.S.A.*, 82:1585-1588 (1985)]. 400,000 recombinants are screened by this procedure. One duplicate positive is plaque purified and the DNA is isolated from a plate lysate of the recombinant bacteriophage designated lambda bP-21. Bacteriophage bP-21 was deposited with the ATCC under accession number ATCC 40310 on March 6, 1987. The bP-21 clone encodes the bovine growth factor designated bBMP-2.

The oligonucleotide hybridizing region of this bBMP-2 clone is localized to an approximately 1.2 kb Sac I restriction fragment which is subcloned into M13 and sequenced by standard techniques. The partial DNA sequence and derived amino acid sequence of this Sac I fragment and the contiguous Hind III-Sac I restriction fragment of bP-21 are shown below in Table II. The bBMP-2 peptide sequence from this clone is 129 amino acids in length and is encoded by the DNA sequence from nucleotide #1 through nucleotide #387. The amino acid sequence corresponding to the tryptic fragment isolated from the bovine bone 28 to 30kd material is underlined in Table II. The underlined portion of the sequence corresponds to tryptic Fragment 3 above from which the oligonucleotide probes for bBMP-2 are designed. The predicted amino acid sequence indicates that tryptic Fragment 3 is preceded by a basic residue (K) as expected considering the specificity of trypsin. The arginine residue encoded by the CGT triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAG) adjacent to it.



TABLE II

	(1)	15	30	45	
5	GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG				
	G H D G K G H P L H R R E K R				
	60	75	90		
	CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG				
	Q A K H K Q R K R L K S S C K				
10	105	120	135		
	AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC				
	R H P L Y V D F S D V G W N D				
	150	165	180		
15	TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG				
	W I V A P P G Y H A F Y C H G				
	195	210	225		
	GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT				
	E C P F P L A D H L N S T N H				
20	240	255	270		
	GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC				
	A I V Q T L V N S V N S K I P				
	385	300	315		
25	AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG				
	K A C C V P T E L S A I S M L				
	330	345	360		
	TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC				
	Y L D E N E K V V L K N Y O D				
30	375	(129)	397	407	
	ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA				
	<u>M V V E G</u> C G C R				
	417	427	437	447	457
	TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC				
35	467	477	487	497	507
	ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA				
	517	527	537	547	557
	AAGAAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA				
40	567	577	587		
	GTGGGAAAA CAAATATTTT AATCAGAGAA TTATT				

EXAMPLE VHuman Bone Inductive FactorsA. hBMP-2: Class I and II

The HindIII-SacI bovine genomic bBMP-2 fragment described in Example IV A. is subcloned into an M13 vector. A <sup>32</sup>p-labeled single-stranded DNA probe is made from a template preparation of this subclone. This probe is used to screen polyadenylated RNAs from various cell and tissue sources.

Polyadenylated RNAs from various cell and tissue sources are electrophoresed on formaldehyde-agarose gels and transferred to nitrocellulose by the method of Toole et al., *supra*. The probe is then hybridized to the nitrocellulose blot in 50% formamide, 5 X SSC, 0.1% SDS, 40 mM sodium phosphate pH6.5, 100 µg/ml denatured salmon sperm DNA, and 5 mM vanadyl ribonucleosides at 42° C overnight and washed at 65° C in 0.2 X SSC, 0.1% SDS. Following autoradiography, a hybridizing band corresponding to an mRNA species of approximately 3.8 kb is detected in the lane containing RNA from the human cell line U-2 OS. The HindIII-SacI fragment is labeled with <sup>32</sup>P by nick translation and

used to screen the nitrocellulose filter replicas of a U-2 OS cDNA library by hybridization in standard hybridization buffer at 65° overnight followed by washing in 1 X SSC, 0.1% SDS at 65°.

This library was constructed by synthesizing cDNA from U-2 OS polyadenylated RNA and cloning into lambda gt10 by established techniques (Toole et al., *supra*). Twelve duplicate positive clones are picked and replated for secondaries. Duplicate nitrocellulose replicas are made of the secondary plates and both sets hybridized to the bovine genomic probe as the primary screening was performed. One set of filters is then washed in 1 X SSC, 0.1% SDS; the other in 0.1 X SSC, 0.1% SDS at 65°.

Two classes of hBMP-2 cDNA clones are evident based on strong (4 recombinants) or weak (7 recombinants) hybridization signals under the more stringent washing conditions (0.1 X SSC, 0.1% SDS). All 11 recombinant bacteriophages are plaque purified, small scale DNA preparations made from plate lysates of each, and the inserts subcloned into pSP65 and into M13 for sequence analysis. Sequence analysis of the strongly hybridizing clones designated hBMP-2 Class I (also known as BMP-2) indicates that they have extensive sequence homology with the sequence given in Table II. These clones are therefore cDNA encoding the human equivalent of the protein encoded by the hBMP-2 gene whose partial sequence is given in Table II. Sequence analysis of the weakly hybridizing recombinants designated hBMP-2 Class II (also known as BMP-4) indicates that they are also quite homologous with the sequence given in Table II at the 3' end of their coding regions, but less so in the more 5' regions. Thus they encode a human protein of similar, though not identical, structure to that above.

Full length hBMP-2 Class I cDNA clones are obtained in the following manner. The 1.5 kb insert of one of the Class II subclones (II-10-1) is isolated and radioactively labeled by nick-translation. One set of the nitrocellulose replicas of the U-2 OS cDNA library screened above (50 filters, corresponding to 1,000,000 recombinant bacteriophage) is rehybridized with this probe under stringent conditions (hybridization at 65° in standard hybridization buffer; washing at 65° in 0.2 X SSC, 0.1% SDS). All recombinants which hybridize to the bovine genomic probe which do not hybridize to the Class II probe are picked and plaque purified (10 recombinants). Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning into M13, sequence analysis indicates that 4 of these represent clones which overlap the original Class I clone. One of these, lambda U2OS-39, contains an approximately 1.5 kb insert and was deposited with the ATCC on June 16, 1987 under accession number 40345. The partial DNA sequence (compiled from lambda U2OS-39 and several other hBMP-2 Class I cDNA recombinants) and derived amino acid sequence are shown below in Table III. Lambda U2OS-39 is expected to contain all of the nucleotide sequence necessary to encode the entire human counterpart of the protein BMP-2 Class I encoded by the bovine gene segment whose partial sequence is presented in Table II. This human cDNA hBMP-2 Class I contains an open reading frame of 1188 bp, encoding a protein of 396 amino acids. This protein of 396 amino acids has a molecular weight of 45kd based on this amino acid sequence. It is contemplated that this sequence represents the primary translation product. The protein is preceded by a 5' untranslated region of 342 bp with stop codons in all frames. The 13 bp region preceding this 5' untranslated region represents a linker used in the cDNA cloning procedure.

TABLE III

5		10	20	30	40	50	60	70
		GTOGACTCTA	GAGTGTGTGT	CAGCACTTGG	CTGGGGACTT	CTTGAACCTG	CAGGGAGAAT	AACITGCGCA
10		80	90	100	110	120	130	140
		CCCCACTTTG	OGGOGGTGOC	TTTGCCCCAG	CGGAGCCTGC	TTGGCCATCT	CGGAGCCCCA	CGGCCCCCTC
15		150	160	170	180	190	200	210
		ACTCCTGGGC	CTTGCCCCGAC	ACTGAGAAGC	TGTTCCAGC	GTGAAAAGAG	AGACTGOGOG	GGGGCAGCC
20		220	230	240	250	260	270	280
		GGGAGAAGGA	GGAGGCAAAG	AAAAGGAACG	GACATTGGT	CTTGCGGCA	GGTCTTTTGA	CCAGATTTT
25		290	300	310	320	330	340	350
		TCCATGTGGA	CGCTCTTTCA	ATGGACGTGT	CCCCGGTGC	TTCTTAGAAG	GACTGGGGTC	TCCTAAAGGT
30		(1)	370	385	400			
		CGAOC	ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC					
			MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val					
35		415	430	445				
		CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC GGC AGG AAG TTC GCG						
		Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala						
40		460	475	490	505			
		GCG GCG TCG TCG GGC GGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG						
		Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu						
45		520	535	550	565			
		TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC						
		Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser						
50		580	595	610				
		AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT GGC AGG CAC TCG GGT						
		Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly						
55		625	640	655	670			
		CAG CCG GGC TCA CCC GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC CGA GGC						
		Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala						
		685	700	715				
		AAC ACT GTG GGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG						
		Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr						

730 745 760 775  
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 5 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 850 865 880  
 10 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 895 910 925 940  
 15 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 20 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 1060 1075 1090 1105  
 25 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 1120 1135 1150  
 30 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 35 1225 1240 1255  
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 1270 1285 1300 1315  
 40 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 1330 1345 1360 1375  
 45 TTT TAC TGC CAC GGA GAA TGC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 1390 1405 1420  
 50 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

55

1435                      1450                      1465                      1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
 5  
                     1495                      1510                      1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GCG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly  
 10  
 1540(396)      1553              1563              1573              1583              1593              1603  
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAA  
 Cys Arg  
 15  
 AAAA

Full-length hBMP-2 Class II human cDNA clones are obtained in the following manner. The 200 bp EcoRI-SacI  
 fragment from the 5' end of the Class II recombinant II-10-1 is isolated from its plasmid subclone, labeled by nick-  
 translation, and hybridized to a set of duplicate nitrocellulose replicas of the U-2 OS cDNA library (25 filters/set; rep-  
 20  
 resenting 500,000 recombinants). Hybridization and washing are performed under stringent conditions as described  
 above. 16 duplicate positives are picked and replated for secondaries. Nitrocellulose filter replicas of the secondary  
 plates are made and hybridized to an oligonucleotide which was synthesized to correspond to the sequence of II-10-1  
 and is of the following sequence:

25                                      **CGGGCGCTCAGGATACTCAAGACCAGTGCTG**

Hybridization is in standard hybridization buffer at 50°C with washing at 50° in 1 X SSC, 0.1% SDS. 14 recombinant  
 bacteriophages which hybridize to this oligonucleotide are plaque purified. Plate stocks are made and small scale  
 bacteriophage DNA preparations made. After subcloning 3 of these into M13, sequence analysis indicates that they  
 represent clones which overlap the original Class II clone. One of these, lambda U2OS-3, was deposited with the ATCC  
 30  
 under accession number 40342 on June 16, 1987. U2OS-3 contains an insert of approximately 1.8 kb. The partial DNA  
 sequence and derived amino acid sequence of U2OS-3 are shown below in Table IV. This clone is expected to contain  
 all of the nucleotide sequence necessary to encode the entire human BMP-2 Class II protein. This cDNA contains an  
 open reading frame of 1224 bp, encoding a protein of 408 amino acids, preceded by a 5' untranslated region of 394  
 bp with stop codons in all frames, and contains a 3' untranslated region of 308 bp following the in-frame stop codon.  
 35  
 The 8 bp region preceding the 5' untranslated region represents a linker used in the cDNA cloning procedure. This  
 protein of 408 amino acids has molecular weight of 47kd and is contemplated to represent the primary translation  
 product.

40

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50

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TABLE IV

5	10	20	30	40	50	60	70
	CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGOGC	GGAGCCCGGC	COGGAAGCTA	GGTGAGTGTC
10	80	90	100	110	120	130	140
	GCATCOGAGC	TGAGGGAGGC	GAGCCTGAGA	CGCGCTGCT	GCTCCGGCTG	AGTATCTAGC	TTGTCTCCCC
15	150	160	170	180	190	200	210
	GATGGGATTC	COGTCCAAGC	TATCTOGAGC	CTGCAGOGCC	ACAGTCCCG	GOOCTCGGCC	AGGTTCACTG
20	220	230	240	250	260	270	280
	CAACCGTTCA	GAGGTCCCA	GGAGCTGCTG	CTGGCGAGCC	CGCTACTGCA	GGGAOCTATG	GAGOCATTCC
25	290	300	310	320	330	340	350
	GTAGTGCCAT	CCCGAGCAAC	GCACGCTGTC	AGCTTCCCTG	AGOCITTCOA	GCAAGTTTGT	TCAAGATTGG
30	360	370	380	390	400	(1)	
	CTGTCAAGAA	TCATGGACTG	TTATTATATG	OCTTGTTTTC	TGTCAAGACA	CC ATG ATT OCT	MET.Ile Pro
35	417	432	447	462			
	GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG						
	Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala						
40	477	492	507				
	AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GGC GAG ATT CAG						
	Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln						
45	522	537	552	567			
	GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC						
	Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe						
50	582	597	612	627			
	GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG OCT AGC AAG						
	Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys						
55	642	657	672				
	AGT GGC GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG						
	Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu						
60	687	702	717	732			
	GAG GAG GAA GAG CAG ATC CAQ AGC ACT GGT CTT GAG TAT OCT GAG CGC CCG GGC						
	Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala						

747 762 777  
 AGC OGG GOC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
 5 792 807 822 837  
 CCA GGG ACC AGT GAA AAC TCT GCT TTT OGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
 10 852 867 882 897  
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT OGG CTC TTC OGG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
 15 912 927 942  
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC OGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
 20 957 972 987 1002  
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
 1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
 25 1062 1077 1092 1107  
 GCG GTC CTT OGC TGG ACC OGG GAG AAG CAG CCA AAC TAT GGG CTA GGC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
 1122 1137 1152 1167  
 30 GTG ACT CAC CTC CAT CAG ACT OGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser  
 1182 1197 1212  
 35 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GOC CAG CTC OGG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
 1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC OGG GGC CAT GOC TTG ACC CGA OGC OGG AGG GOC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys  
 40 1287 1302 1317  
 OGT AGC OCT AAG CAT CAC TCA CAG OGG GOC AGG AAG AAG AAT AAG AAC TGC OGG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 1332 1347 1362 1377  
 45 OGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
 1392 1407 1422 1437  
 50 GOC CCA CCA GGC TAC CAG GOC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

55

1452                      1467                      1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GOC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser  
 5  
 1497                      1512                      1527                      1542  
 GTC AAT TOC AGT ATC CCC AAA GOC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 10  
 1557                      1572                      1587  
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 1602                      1617                      (408)                      1636                      1646                      1656  
 ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCTTGA GGATAGACAG  
 15  
 MET Val Val Glu Gly Cys Gly Cys Arg  
 1666                      1676                      1686                      1696                      1706                      1716                      1726  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA TOCACTCACC CACACACTAC  
 20  
 1736                      1746                      1756                      1766                      1776                      1786                      1796  
 ACAGACTGCT TOCTTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAATAA AATGGAAAAA ATCCTTAAAC  
 25  
 1806                      1816                      1826                      1836                      1846                      1856                      1866  
 AATCACTTGG ACCTTATTTA TGACTTTAAG TGCAATGTT TTGACCATAT TGATCATATA TTTTGACAAA  
 30  
 1876                      1886                      1896                      1906                      1916                      1926                      1936  
 ATATATTTTAT AACTACGTAT TAAAAGAAAA AATAAAATG AGTCATTATT TTAAAAAATA AAAAAAACT  
 35  
 1946  
 CTAGAGTCGA CGGAATTC

The sequences of BMP-2 Class I and II as shown in Tables II, III IV and have significant homology to the beta (B) and beta (A) subunits of the inhibins. The inhibins are a family of hormones which are presently being investigated for use in contraception. See, A. J. Mason et al, *Nature*, 318:659-663 (1985). To a lesser extent they are also homologous to Mullerian inhibiting substance (MIS), a testicular glycoprotein that causes regression of the Mullerian duct during development of the male embryo and transforming growth factor-beta (TGF-b) which can inhibit or stimulate growth of cells or cause them to differentiate. Furthermore, the sequence of Table IV encoding hBMP-2 Class II has significant homology to the *Drosophila* decapentaplegic (DPP-C) locus transcript. See, J. Massague, *Cell*, 49:437-438 (1987); R. W. Padgett et al, *Nature*, 325:81-84 (1987); R.L. Cate et al, *Cell* 45: 685-698 (1986). It is considered possible therefore that BMP-2 Class II is the human homolog of the protein made from this transcript form this developmental mutant locus.

#### EXAMPLE VI

##### Expression of Bone Inductive Factors.

In order to produce bovine, human or other mammalian bone inductive factors, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells by conventional genetic engineering techniques.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Tables II, III AND IV or other modified sequences and known vectors, such as pCD [Okayama et al., *Mol. Cell Biol.*, 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., *EMBO J.*, 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells can result in expression of osteoinductive factors. One skilled in the art could manipulate the sequences of Tables II, III and IV by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For ex-



ample, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences therefrom or altering nucleotides therein by other known techniques). The modified bone inductive factor coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and bone inductive factor expressed thereby. For a strategy for producing extra-cellular expression of bone inductive factor in bacterial cells., see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [see, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application W086/00639 and European patent application EPA 123,289].

A method for producing high levels of an osteoinductive factor of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous bone inductive factor gene. The heterologous gene can be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, J. Mol. Biol., 159:601-629 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a bone inductive factor of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A) 3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5: 1750 (1983). Transformants are cloned, and biologically active bone inductive factor expression is monitored by rat bone formation assay. Bone inductive factor expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other bone inductive factors.

Alternatively, the human gene is expressed directly, as described above. Active bone inductive factor may be produced in bacteria or yeast cells. However the presently preferred expression system for biologically active recombinant human bone inductive factor is stably transformed CHO cells.

As one specific example, to produce the human bone inductive factor (hBMP-1) of Example V, the insert of U2OS-1 is released from the vector arms by digestion with Sal I and subcloned into the mammalian expression vector pMT2CX digested with Xho I. Plasmid DNA from this subclone is transfected into COS cells by the DEAE-dextran procedure [Sompayrac and Danna PNAS 78:7575-7578 (1981); Luthman and Magnusson, Nucl.Acids Res. 11: 1295-1308 (1983)]. Serum-free 24 hr. conditioned medium is collected from the cells starting 40-70 hr. post-transfection.

The mammalian expression vector pMT2 Cla-Xho (pMT2 CX) is a derivative of p91023 (b) (Wong et al., Science 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a XhoI site for insertion of cDNA clones. The functional elements of pMT2 Cla-Xho have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 Cla-Xho is obtained by EcoRI digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRI digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform E. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2CX is then constructed by digesting pMT2 with Eco RV and XbaI, treating the digested DNA with Klenow fragment of DNA polymerase I, and ligating Cla linkers (NEBiolabs, CATCGATG). This removes bases 2266 to 2421 starting from the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. Plasmid DNA is then digested with EcoRI, blunted as above, and ligated to an EcoRI adapter,

5' PO<sub>4</sub>-AATTCCTCGAGAGCT 3'

3' GGAGCTCTCGA 5'

digested with XhoI, and ligated, yielding pMT2 Cla-Xho, which may then be used to transform E. coli to ampicillin resistance. Plasmid pMT2 Cla-Xho DNA may be prepared by conventional methods.

Example VIIBiological Activity of Expressed Bone Inductive Factor

## A. BMP-1

To measure the biological activity of the expressed bone inductive factor. (hBMP-1) obtained in Example VI above. The factor is partially purified on a Heparin Sepharose column. 4 ml of transfection supernatant from one 100 mm dish is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a 1.1 ml Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including BMP-1, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for *in vivo* bone and cartilage formation as previously described in Example III. A mock transfection supernatant fractionation is used as a control.

The implants containing rat matrix to which specific amounts of human BMP-1 have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated.

Addition of human BMP-1 to the matrix material resulted in formation of cartilage-like nodules at 7 days post implantation. The chondroblast-type cells were recognizable by shape and expression of metachromatic matrix. The amount of activity observed for human BMP-1 was dependent upon the amount of human BMP-1 protein added to the matrix. Table IX illustrates the dose-response relationship of human BMP-1 protein to the amount of bone induction observed.

Table IX

IMPLANT NUMBER	AMOUNT USED (equivalent of ml transfection media)	HISTOLOGICAL SCORE
876-134-1	10 BMP-1	C+2
876-134-2	3 BMP-1	C+1
876-134-3	1 BMP-1	C+/-
876-134-4	10 MOCK	C-
876-134-5	3 MOCK	C -
876-134-6	1 MOCK	C -

Cartilage (c) activity was scored on a scale from 0(-) to 5.

Similar levels of activity are seen in the Heparin Sepharose fractionated COS cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers. Further, in a rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic activity.

The procedures described above may be employed to isolate other bone inductive factors of interest by utilizing the bovine bone inductive factors and/or human bone inductive factors as a probe source. Such other bone inductive factors may find similar utility in, inter alia, fracture repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

## Claims

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A gene encoding human BMP-2 comprising the following DNA sequence:

10 20 30 40 50 60 70  
 GTGACTCTA GAGTGTGIGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACITGGCCA  
 5  
 80 90 100 110 120 130 140  
 CCCACTTTG GCGGGTGGC TTTGCCCCAG GCGAGCTGC TTGOCATCT CCGAGCCCCA CCGCCCCCTCC  
 10  
 150 160 170 180 190 200 210  
 ACTOCTGGC CTTGCCCGAC ACTGAGAAGC TGTTCOCAGC GTGAAAAGAG AGACTGGCGG GCGGCAACC  
 15  
 220 230 240 250 260 270 280  
 GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTGGT CTTTGGCCA GGTCTTTTGA CCAGAGTTTT  
 20  
 290 300 310 320 330 340 350  
 TOCATGTGGA CGCTCTTCA ATGGAAGTGT CCCGGGTGC TTCTAGAAG GACTGGGTC TCCTAAAGGT  
 (1) 370 385 400  
 OGACC ATG GTG GGC GGG ACC GCG TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 25  
 415 430 445  
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC GCG AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 30  
 460 475 490 505  
 GCG GCG TCG TCG GGC GCG CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 35  
 520 535 550 565  
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 580 595 610  
 AGG GAC GCG GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CCG AGG CAC TCG GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 40  
 625 640 655 670  
 CAG CCG GGC TCA CCC GCG CCA GAC CAC CCG TTG GAG AGG GCA GCG AGC CGA GCG  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala  
 45  
 685 700 715  
 AAC ACT GTG GCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr  
 50  
 55

730 745 760 775  
 AGT GGG AAA ACA ACC OGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 5  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC OGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 10  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC OGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 15  
 895 910 925 940  
 OCT GCA ACA GOC AAC TOG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 20  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 25  
 1000 1015 1030 1045  
 OGG TGG ACT GCA CAG GGA CAC GOC AAC CAT GGA TTC GTG GTG GAA GTG GCC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 30  
 1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TOC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 35  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 40  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GCC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 45  
 1225 1240 1255  
 AAA CAG OGG AAA OGC CTT AAG TOC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 50  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 55  
 1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 1390 1405 1420  
 AAT CAT GCC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435 1450 1465 1480  
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
5  
1495 1510 1525  
AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG  
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly  
10  
1540(396) 1553 1563 1573 1583 1593 1603  
TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTATG AAAAAAGAAA  
Cys Arg

15 AAAA

2. A gene encoding human BMP-2 having the amino acid sequence given in claim 1.
3. A gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:
  - (a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code;
  - (b) which hybridises with a DNA sequence of claim 1 or section (a), above; or
  - (c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not.
4. The DNA sequence of claim 3, which is a genomic DNA sequence.
5. The DNA sequence of claim 3, which is a cDNA sequence.
6. A gene encoding bovine BMP-2 comprising the following DNA sequence:

(1) 15 30 45  
GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
G H D G K G H P L H R R E K R

5 CAA GCA AAA CAC AAA CAG CGG AAA CGC 75 90  
Q A K H K Q R K R L K S S C K

10 AGA CAC CCT TTA 105 120 135  
R H P L Y V D F S D V G W N D

15 TGG ATC GTT GCA 150 165 180  
W I V A P P G Y H A F Y C H G

20 GAG TGC CCT TTT 195 210 225  
E C P F P L A D H L N S T N H

GCC ATT CTC CAA 240 255 270  
A I V Q T L V N S V N S K I P

25 AAG GCA TGC TGT 385 300 315  
K A C C V P T E L S A I S M L

30 TAC CTT GAT GAG 330 345 360  
Y L D E N E K V V L K N Y Q D

35 ATG GTT GTC GAG 375 (129) 397 407  
M V V E G C G C R TAGCACAGCA AAATAAAATA

417 427 437 447 457  
TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

40 467 477 487 497 507  
ACTTTAATAT TTCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

517 527 537 547 557  
AAGAAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA

45 567 577 587  
GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

- 50 7. A gene encoding bovine BMP-2 containing the amino acid sequence of claim 6.
8. A gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:
- 55 (a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;  
(b) which hybridise with a DNA sequence of claim 7 or section (a), above; or  
(c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not.

9. The DNA sequence of claim 8, which is a genomic DNA sequence.

10. The DNA sequence of claim 8, which is a cDNA sequence.

5 11. A gene encoding human BMP-4 comprising the following DNA sequence:

10  
 15  
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 335  
 340  
 345  
 350  
 355  
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 365  
 370  
 375  
 380  
 385  
 390  
 395  
 400  
 (1)  
 CTCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGOGC GGAGOOOOGC CCGGAGCTA GGTGAGTGTG  
 GCATOOGAGC TGAGGGAOGC GAGCCTGAGA GGOOGCTGCT GCTOOGGCTG AGTATCTAGC TTGTCTCOOC  
 GATGGGATTG CCGTCCAAGC TATCTOGAGC CTGCAGOGCC ACAGTCCOOG GCOCTOGOC AGGTTTACTG  
 CAACCGTTCA GAGGTCCOOC GGAGCTGCTG CTGGGAGOC OGCTACTGCA GGGAOCTATG GAGCCATTCC  
 GTAGTGCAT CCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG  
 CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTITTC TGTCAGACA CC ATG ATT OCT  
 MET Ile Pro

417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GOG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 5  
 477 492 507  
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 10  
 522 537 552 567  
 GGC CAC GOG GGA GGA OGC OGC TCA GGG CAG AGC CAT GAG CTC CTG OGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 15  
 582 597 612 627  
 GAG GOG ACA CTT CTG CAG ATG TTT GGG CTG OGC OGC OGC OGC CAG OCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 20  
 642 657 672  
 AGT GCC GTC ATT OGC GAC TAC ATG OGC GAT CTT TAC OGC CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 25  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG OGC OGC GCC  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
 30  
 747 762 777  
 AGC OGC GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
 35  
 792 807 822 837  
 OCA GGG ACC AGT GAA AAC TCT GCT TTT OGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
 40  
 852 867 882 897  
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT OGC CTC TTC OGC GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
 45  
 912 927 942  
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC OGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
 50  
 957 972 987 1002  
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
 55  
 1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA OGG TGG GAA ACT TTT GAT GTG AGC OCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
 1062 1077 1092 1107  
 GOG GTC CTT OGC TGG ACC OGC GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
 1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT OGC ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser



1182 1197 1212  
 OGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GOC CAG CTC OGG COC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
 5  
 1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC OGG GGC CAT GCC TTG AOC CGA OGC OGG AGG GOC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys  
 10  
 1287 1302 1317  
 OGT AGC OCT AAG CAT CAC TCA CAG OGG GOC AGG AAG AAG AAT AAG AAC TGC OGG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 1332 1347 1362 1377  
 OGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
 15  
 1392 1407 1422 1437  
 GOC CCA OCA GGC TAC CAG GOC TTC TAC TGC CAT GGC GAC TGC COC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu  
 20  
 1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GOC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser  
 25  
 1497 1512 1527 1542  
 GTC AAT TCC AGT ATC COC AAA GOC TGT TGT GTG COC ACT GAA CTG AGT GOC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 1557 1572 1587  
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 30  
 1602 1617 (408) 1636 1646 1656  
 ATG GTA GTA GAG GGA TGT GGG TGC OGC TGAGATCAGG CAGTCCTTGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
 35  
 1666 1676 1686 1696 1706 1716 1726  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CAAGTTCOCA TCCACTCAAC CACACACTAC  
 1736 1746 1756 1766 1776 1786 1796  
 ACAGACTGCT TCCCTATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATCGAAAAA ATCCTAAAC  
 40  
 1806 1816 1826 1836 1846 1856 1866  
 ATTCACCTTG ACCCTATTTA TGACTTTTAC TGCAATGTT TTGACCATAT TGATCATATA TTTTGACAAA  
 45  
 1876 1886 1896 1906 1916 1926 1936  
 ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAACT  
 50  
 1946  
 CTAGAGTCGA CGGAATTC  
 55

12. A gene encoding human BMP-4 having the amino acid sequence given in claim 11.

13. A gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:

- (a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;  
 (b) which hybridises with a DNA sequence of claim 11 or section (a), above; or  
 (c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not.

14. The DNA sequence of claim 13, which is a genomic DNA sequence.

15. The DNA sequence of claim 13, which is a cDNA sequence.

16. A vector containing the gene or DNA sequence of any one of claims 1 to 15 in operative association with an expression control sequence.

17. A cell transformed with a vector of claim 16.

18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.

19. The cell of claim 18 which is a CHO cell.

20. A protein exhibiting properties of BMP-2 which is encoded by a gene or DNA sequence of any one of claims 1 to 10.

21. A protein exhibiting properties of BMP-2, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 1 to 10, and recovering said protein from said culture medium.

22. A protein exhibiting properties of BMP-4 which is encoded by a gene or DNA sequence of any one of claims 11 to 15.

23. A protein exhibiting properties of BMP-4, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 11 to 15, and recovering said protein from said culture medium.

24. A process for producing the protein of claims 21 or 23, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.

25. A pharmaceutical composition comprising the proteins of any one of claims 20 to 23, individually or in combination, and a pharmaceutically acceptable vehicle.

26. The pharmaceutical composition of claim 25, further comprising a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.

27. The pharmaceutical composition of claim 26, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.

28. Use of a protein of any one of claims 20 to 23, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

#### Claims for the following Contracting State : AT

1. A process for the preparation of a gene encoding human BMP-2 comprising the following DNA sequence:

10 20 30 40 50 60 70  
 GTGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGGCA  
 5  
 80 90 100 110 120 130 140  
 CCCCCTTTG OGGGGTGGC TTGCCCCAG CGGAGCTGTC TTGGCATCT OOGAGOOCCA OGGOOCTCC  
 10  
 150 160 170 180 190 200 210  
 ACTOCTGGC CTTGCOOGAC ACTGAGACGC TGTTOCCAGC GTGAAAAGAG AGACTGOGOG GCGGGCAOC  
 15  
 220 230 240 250 260 270 280  
 GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTGGT CCTTGOGCCA GGTCTTTGA CCAGAGTTT  
 290 300 310 320 330 340 350  
 TCCATGTGA CGCTCTTCA ATGGAOGTGT CCGOGGTGC TTCTTAGACG GACTGOGGTC TCTAAAGGT  
 20  
 (1) 370 385 400  
 OGACC ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT OCC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 25  
 415 430 445  
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC GCG AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 30  
 460 475 490 505  
 GCG GCG TCG TCG GGC GCG CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 35  
 520 535 550 565  
 TTC GAG TTG GCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC AOC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 580 595 610  
 AGG GAC GCG GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CCG AGG CAC TCG GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 40  
 625 640 655 670  
 CAG CCG GGC TCA CCC GCG CCA GAC CAC GCG TTG GAG AGG GCA GCG AGC CGA GCG  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala  
 45  
 685 700 715  
 AAC ACT GTG GCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr  
 50  
 55

730 745 760 775  
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC OCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 5  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 10  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 15  
 895 910 925 940  
 OCT GCA ACA GOC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 20  
 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GOC AAC CAT GGA TTC GTG GTG GAA GTG GOC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 25  
 1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TOC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 30  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 35  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GOC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 1225 1240 1255  
 AAA CAG CGG AAA CGC CTT AAG TOC AGC TGT AAG AGA CAC OCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 40  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GOC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 45  
 1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TOC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 50  
 1390 1405 1420  
 AAT CAT GOC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys  
 55

1435                      1450                      1465                      1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TGG ATG CTG TAC CTT GAC GAG  
 5 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
  
                     1495                      1510                      1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly  
 10  
 1540(396)            1553            1563            1573            1583            1593            1603  
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA  
 Cys Arg  
 15  
 AAAA ,

wherein said process comprises the following steps:

- 20            a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,  
               b) isolating positive clones, and  
               c) isolating the DNA-inserts from said clones.
- 25            2. The process according to claim 1, wherein the gene encodes human BMP-2 having the amino acid sequence given in claim 1.
- 30            3. A process for the preparation of a gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:
  - a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code;
  - b) which hybridizes with a DNA sequence of claim 1 or section (a), above; or
  - c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not,
- 35            wherein said process comprises standard techniques of molecular biology.
- 40            4. The process according to claim 3, wherein the DNA sequence is a genomic DNA sequence.
- 50            5. The process according to claim 3, wherein the DNA sequence is a cDNA sequence.
- 55            6. A process for the preparation of a gene encoding bovine BMP-2 comprising the following DNA sequence:

(1) 15 30 45  
 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
 G H D G K G H P L H R R E K R  
 5  
 60 75 90  
 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG  
 Q A K H K Q R K R L K S S C K  
 10  
 105 120 135  
 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC  
 R H P L Y V D F S D V G W N D  
 15  
 150 165 180  
 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG  
 W I V A P P G Y H A F Y C H G  
 20  
 195 210 225  
 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT  
 E C P F P L A D H L N S T N H  
 25  
 240 255 270  
 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC  
 A I V Q T L V N S V N S K I P  
 30  
 385 300 315  
 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG  
 K A C C V P T E L S A I S M L  
 35  
 330 345 360  
 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC  
 Y L D E N E K V V L K N Y Q D  
 375 (129) 397 407  
 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA  
 M V V E G C G C R  
 40  
 417 427 437 447 457  
 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC  
 467 477 487 497 507  
 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA  
 45  
 517 527 537 547 557  
 AAGAAAAACA CAGCTATTTT GAAAACATA TTTATATCTA CCGAAAAGAA  
 567 577 587  
 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,

50 wherein said process comprises the following steps:

- 55
- a) screening a gene library constructed from bovine liver DNA or cDNA with a labelled probe designed on the basis of the amino acid sequence of a fragment of bBMP-2,
  - b) isolating positive clones, and
  - c) isolating the DNA-inserts from said clones.

7. The process according to claim 6, wherein the gene encodes bovine BMP-2 having the amino acid sequence of claim 6.

8. A process for the preparation of a gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:

- 5           a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;  
          b) which hybridize with a DNA sequence of claim 7 or section a), above; or  
          c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not,

10           wherein said process comprises standard techniques of molecular biology.

9. The process according to claim 8, wherein the DNA sequence is a genomic DNA sequence.

10. The process according to claim 8, wherein the DNA sequence is a cDNA sequence.

- 15 11. A process for the preparation of a gene encoding human BMP-4 comprising the following DNA sequence:

20

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50

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10 20 30 40 50 60 70  
 CTCTAGAGGG CAGAGGAGCA GGGAGGGAGG GAAGGAGGCG GGAGCCCGGC CCGGAAGCTA GGTGAGTGTG  
 5  
 80 90 100 110 120 130 140  
 GCATCCGAGC TGAGGGAGCG GAGCCTGAGA CGCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCG  
 10  
 150 160 170 180 190 200 210  
 GATGGGATTG CCGTCCAGC TATCTGAGC CTGCAGCGC ACAGTCCCG GCGCTCGCC AGGTTTCACTG  
 15  
 220 230 240 250 260 270 280  
 CAACCGTTCA GAGGTCCCA GGAGCTGCTG CTGGCGAGCG CGCTACTGCA GGGACCTATG GAGCATTC  
 290 300 310 320 330 340 350  
 GTAGTGCAT CCGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG  
 20  
 360 370 380 390 400 (1)  
 CTGTCAAGAA TCATGGACTG TTATTATATG CCTGTGTTTC TGTCAGACA CC ATG ATT OCT  
 MET Ile Pro...  
 25  
 417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 30  
 477 492 507  
 AGC CAT GCT AGT TTG ATA CCT GAG ACG GGG AAG AAA AAA GTC GCG GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 35  
 522 537 552 567  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG GCG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG OCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 40  
 642 657 672  
 AGT GCG GTC ATT CCG GAC TAC ATG CCG GAT CTT TAC CCG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 45  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCG  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
 50  
 55



5                   747                   762                   777  
 AGC CCG GGC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
  
 792                   807                   822                   837  
 CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
  
 10                   852                   867                   882                   897  
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CCG CTC TTC CCG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
  
 15                   912                   927                   942  
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
  
 20                   957                   972                   987                   1002  
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
  
 25                   1017                   1032                   1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CCG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
  
 1062                   1077                   1092                   1107  
 GCG GTC CTT CCG TGG ACC CCG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
  
 30                   1122                   1137                   1152                   1167  
 GTG ACT CAC CTC CAT CAG ACT CCG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser  
  
 35                   1182                   1197                   1212  
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GGC CAG CTC CCG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
  
 40                   1227                   1242                   1257                   1272  
 ACC TTT GGC CAT GAT GGC CCG GGC CAT GCC TTG ACC CGA CCG CCG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys  
  
 45                   1287                   1302                   1317  
 CGT AGC CCT AAG CAT CAC TCA CAG CCG GCC AGG AAG AAG AAT AAG AAC TGC CCG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
  
 1332                   1347                   1362                   1377  
 CCG CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
  
 50                   1392                   1407                   1422                   1437  
 GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Glv Aso Cys Pro Phe Pro Leu  
  
 55                   1452                   1467                   1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497                      1512                      1527                      1542  
 GTC AAT TOC AGT ATC CAC AAA GGC TGT TGT GTG CAC ACT GAA CTG AGT GCC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 5  
                     1557                      1572                      1587  
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 10  
 1602                      1617                      (408)                      1636                      1646                      1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CAC TGAGATCAGG CAGTCTTGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
                     1666                      1676                      1686                      1696                      1706                      1716                      1726  
 15 ATATACACAC CACACACACA CACACATAC ACCACACACA CAGTTTCCCA TOCACTCACC CACACACTAC  
                     1736                      1746                      1756                      1766                      1776                      1786                      1796  
 20 ACAGACTGCT TCCTTATAGC TGGACTTTTA TTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCTTAAAC  
                     1806                      1816                      1826                      1836                      1846                      1856                      1866  
 25 ATTCAOCTTG AOCTTATTTA TGACTTTTAC TGCAATGTT TTGACCATAT TGATCATATA TTTTGACAAA  
                     1876                      1886                      1896                      1906                      1916                      1926                      1936  
 30 ATATATTTTAT AACTACGTAT TAAAAGAAAA AAATAAATG AGTCATTATT TTAACAAAAA AAAAAAACT  
 1946  
 CTAGAGTGA CGGAATTC ,

wherein said process comprises the following steps:

- 35 a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,  
 b) isolating positive clones, and  
 c) isolating the DNA-inserts from said clones.
- 40 12. The process according to claim 11, wherein the gene encodes human BMP-4 having the amino acid sequence given in claim 11.
- 45 13. A process for the preparation of a gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:
- 50 a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;  
 b) which hybridizes with DNA sequence of claim 11 or section a), above; or  
 c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not,
- wherein said process comprises standard techniques of molecular biology.
14. The process according to claim 13, wherein the DNA sequence is a genomic DNA sequence.
- 55 15. The process according to claim 13, wherein the DNA sequence is a cDNA sequence.
16. A vector containing the gene or DNA sequence prepared according to any one of claims 1 to 15 in operative association with an expression control sequence.

17. A cell transformed with a vector of claim 16.
18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
- 5 19. The cell of claim 18 which is a CHO cell.
20. A process for the preparation of a protein exhibiting properties of BMP-2, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 1 to 10, and recovering said protein from said culture medium.
- 10 21. A process for the preparation of a protein exhibiting properties of BMP-4, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 11 to 15, and recovering said protein from said culture medium.
- 15 22. A process for producing a protein exhibiting properties of BMP-2 or BMP-4, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.
- 20 23. A process for the preparation of a pharmaceutical composition comprising combining the proteins prepared according to any one of claims 20 to 22, individually or in combination with a pharmaceutically acceptable vehicle.
24. The process according to claim 23, wherein said pharmaceutical composition further comprises a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.
- 25 25. The process according to claim 24, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.
- 30 26. Use of a protein prepared according to any one of claims 20 to 22, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

#### Patentansprüche

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Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Menschliches BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

40

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10 20 30 40 50  
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG  
 5 60 70 80 90 100  
 CAGGGAGAAT AACTTGCGCA CCCCACCTTG CGCCGGTGCC TTTGCCCCAG  
 110 120 130 140 150  
 CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCTCC ACTCCTCGGC  
 10 160 170 180 190 200  
 CTTGCCCCGAC ACTGAGACGC TGTTCCTCAGC GTGAAAAGAG AGACTGCGCG  
 210 220 230 240 250  
 15 GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT  
 260 270 280 290 300  
 CCTTGCGCCA GGTCCTTTGA CCAGAGTTTT TCCATGTGGA CGCTCTTTCA  
 20 310 320 330 340 350  
 ATGGACGTGT CCCC GCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT  
 (1) 370 385 400  
 25 CGAOC ATG GTG GOC GGG AOC CGC TGT CTT CTA GOG TTG CTG CTT OOC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 415 430 445  
 30 CTC CTG GGC GGC GOG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GOG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 460 475 490 505  
 GOG GOG TOG TOG GGC CGC OOC TCA TOC CAG OOC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 35 520 535 550 565  
 TTC GAG TTG OGG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA OOC AOC OOC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 40 580 595 610  
 AGG GAC GOC GTG GTG OOC OOC TAC ATG CTA GAC CTG TAT CGC AGG CAC TOG GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 45  
 50  
 55

625 640 655 670  
 CAG CCG GGC TCA CCG GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC CCA GGC  
 5 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala

685 700 715  
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 10 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730 745 760 775  
 AGT GGG AAA ACA ACC CCG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCG ACG GAG  
 15 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu

790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala

850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 20 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys

895 910 925 940  
 OCT GCA ACA GGC AAC TCG AAA TTC CCG GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 25 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu

955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCG GCT GTG ATG  
 30 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET

1000 1015 1030 1045  
 CCG TGG ACT GCA CAG CGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC  
 35 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His

1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 40 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu

1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly

1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC  
 45 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His

1225 1240 1255  
 AAA CAG CCG AAA CCG CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 50 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp

1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCG CCG GGG TAT CAC GGC  
 55 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala

1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 5  
 1390 1405 1420  
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys  
 10  
 1435 1450 1465 1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
 15  
 1495 1510 1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly  
 20  
 1540(396) 1553 1563 1573 1583 1593 1603  
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA  
 Cys Arg  
 25  
 AAAA

2. Gen, das menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist.
3. Gen, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt,  
 die:
  - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;
  - (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder
  - (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
4. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
5. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
6. Rinder-BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

(1) 15 30 45  
 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
 G H D G K G H P L H R R E K R  
 5  
 60 75 90  
 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG  
 Q A K H K Q R K R L K S S C K  
 10  
 105 120 135  
 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC  
 R H P L Y V D F S D V G W N D  
 15  
 150 165 180  
 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG  
 W I V A P P G Y H A F Y C H G  
 20  
 195 210 225  
 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT  
 E C P F P L A D H L N S T N H  
 25  
 240 255 270  
 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC  
 A I V Q T L V N S V N S K I P  
 30  
 385 300 315  
 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG  
 K A C C V P T E L S A I S M L  
 35  
 330 345 360  
 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC  
 Y L D E N E K V V L K N Y O D  
 40  
 375 (129) 397 407  
 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAATA  
 M V V E G C G C R  
 45  
 417 427 437 447 457  
 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC  
 467 477 487 497 507  
 ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAAATGG AATGGAGAAA  
 517 527 537 547 557  
 AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA  
 567 577 587  
 GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT ,  
 50

7. Gen, das Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 enthält.

8. Gen, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

(b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder

(c) Fragmente, allelische oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht.

5

9. DNA-Sequenz nach Anspruch 8, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.

10. DNA-Sequenz nach Anspruch 8, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.

10 11. Menschliches BMP-4 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

10	20	30	40	50
CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCGGC

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 15  
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 25  
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 35  
 40  
 45  
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 55

60 70 80 90 100  
 CCGGAAGCTA GGTGAGTGTG GCATCCGAGC TGAGGGGACGC GAGCCTGAGA  
 110 120 130 140 150  
 CGCCGCTGCT GCTCCGGCTG AGTATCTAGC TTGTCTCCCC GATGGGATTC  
 160 170 180 190 200  
 CCGTCCAAGC TATCTCGAGC CTGCAGCGCC ACAGTCCCCG GCCCTCGCCC  
 210 220 230 240 250  
 AGGTTCACTG CAACCGTTCA GAGGTCCCCA GGAGCTGCTG CTGGCGAGCC  
 260 270 280 290 300  
 CGCTACTGCA GGGACCTATG GAGCCATTCC GTAGTGCCAT CCCGAGCAAC  
 310 320 330 340 350  
 GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG  
 360 370 380 390 400  
 CTGTCAAGAA TCATGGACTG TTATTATATG CCTTGTTTTT TGTCAAGACA  
 (1)  
 CC ATG ATT CCT  
 MET Ile Pro

417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 477 492 507  
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GGC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 522 537 552 567  
 GGC CAC GCG GGA GGA GGC GGC TCA GGG CAG AGC CAT GAG CTC CTG GCG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG GCG GCG CCG CAG OCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 642 657 672  
 AGT GCG GTC ATT CCG GAC TAC ATG CCG GAT CTT TAC CCG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG GCG CCG GCG  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
 747 762 777  
 AGC CCG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

792 807 822 837  
 OCA GGG ACC AGT GAA AAC TCT GCT TTT OGT TTC CTC TTT AAC CTC AGC AGC ATC  
 5 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

852 867 882 897  
 OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG CAG GTG  
 10 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

912 927 942  
 GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC OGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

957 972 987 1002  
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 15 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC OCT  
 20 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1062 1077 1092 1107  
 GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GGC ATT GAG  
 25 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 30 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1182 1197 1212  
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GOC CAG CTC CGG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val

1227 1242 1257 1272  
 AOC TTT GGC CAT GAT GGC CGG GGC CAT GOC TTG ACC CGA CGC CGG AGG GOC AAG  
 35 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys

1287 1302 1317  
 OGT AGC OCT AAG CAT CAC TCA CAG CGG GOC AGG AAG AAG AAT AAG AAC TGC CGG  
 40 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg

1332 1347 1362 1377  
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 45 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val

1392 1407 1422 1437  
 GOC CCA CCA GGC TAC CAG GOC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 50 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu

1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GOC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

55

1497                      1512                      1527                      1542  
 GTC AAT TOC AGT ATC CCC AAA GOC TGT TGT GTG CCC ACT GAA CTG AGT GOC ATC  
 5 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
  
                     1557                      1572                      1587  
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 10  
 1602                      1617                      (408)                      1636                      1646                      1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCITGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
 15  
  
                     1666                      1676                      1686                      1696                      1706  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA  
 20  
                     1716                      1726                      1736                      1746                      1756  
 TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA  
  
                     1766                      1776                      1786                      1796                      1806  
 TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG  
 25  
                     1816                      1826                      1836                      1846                      1856  
 ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA  
  
                     1866                      1876                      1886                      1896                      1906  
 TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG  
 30  
                     1916                      1926                      1936                      1946  
 AGTCATTATT TTAAAAAAAA AAAAAAACT CTAGAGTCGA CGGAATTC

- 35 12. Gen, das menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.
13. Gen, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:
- 40 (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-sequenz nach Anspruch 11 unterscheidet;
- (b) mit einer DNA-Sequenz nach Anspruch 11 oder nach vorstehendem Absatz (a) hybridisiert; oder
- 45 (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
14. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
- 50 15. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.
- 55 17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.
18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.

19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.
20. Protein, das Eigenschaften von BMP-2 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 codiert ist.
21. Protein, das Eigenschaften von BMP-2 aufweist, das erhältlich ist durch die Schritte
- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 umfaßt, und
  - Gewinnen des Proteins aus dem Kulturmedium.
22. Protein, das Eigenschaften von BMP-4 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 codiert ist.
23. Protein, das Eigenschaften von BMP-4 aufweist, das erhältlich ist durch die Schritte
- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 umfaßt und
  - Isolieren des Proteins aus dem Kulturmedium.
24. Verfahren zur Herstellung des Proteins nach Anspruch 21 oder 23, umfassend die Schritte
- Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
  - Gewinnen des Proteins aus dem Kulturmedium.
25. Arzneimittel, dadurch gekennzeichnet, daß es, einzeln oder in Kombination, die Proteine nach einem der Ansprüche 20 bis 23 und einen pharmakologisch verträglichen Träger umfaßt.
26. Arzneimittel nach Anspruch 25, dadurch gekennzeichnet, daß es ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.
27. Arzneimittel nach Anspruch 26, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.
28. Verwendung des Proteins nach einem der Ansprüche 20 bis 23, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

**Patentansprüche für folgenden Vertragsstaat : AT**

1. Verfahren zur Herstellung eines menschlichen BMP-2 codierenden Gens, das die nachfolgende DNA-Sequenz umfaßt:

10 20 30 40 50  
 GTCGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG  
 5 60 70 80 90 100  
 CAGGGAGAAT AACTTGCGCA CCCCACCTTG CGCCGGTGCC TTTGCCCCAG  
 110 120 130 140 150  
 CGGAGCCTGC TTCGCCATCT CCGAGCCCCA CCGCCCCCTCC ACTCCTCGGC  
 10 160 170 180 190 200  
 CTTGCCCCGAC ACTGAGACGC TGTTCCTCAGC GTGAAAAGAG AGACTGCGCG  
 210 220 230 240 250  
 15 GCCGGCACCC GGGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTCGGT  
 260 270 280 290 300  
 CCTTGCGCCA GGTCCCTTTGA CCAGAGTTTT TCCATGTGGA CGCTCTTTCA  
 20 310 320 330 340 350  
 ATGGACGTGT CCCC GCGTGC TTCTTAGACG GACTGCGGTC TCCTAAAGGT  
 (1) 370 385 400  
 25 OGACC ATG GTG GGC GGG AOC CGC TGT CTT CTA GCG TTG CTG CTT OOC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 415 430 445  
 CTC CTG GGC GGC GCG GCT GGC CTC GTT CCG GAG CTG GGC CGC AGG AAG TTC GCG  
 30 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 460 475 490 505  
 GCG GCG TCG TCG GGC CGC OOC TCA TCC CAG OOC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 35 520 535 550 565  
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA OOC AOC OOC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 580 595 610  
 40 AGG GAC GGC GTG GTG OOC OOC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 45  
 50  
 55

625 640 655 670  
 CAG CCG GGC TCA CCG GGC CCA GAC CAC CGG TTG GAG AGG GCA GGC AGC CGA GGC  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala  
 5  
 685 700 715  
 AAC ACT GTG GGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr  
 10  
 730 745 760 775  
 AGT GGG AAA ACA ACC GGC AGA TTC TTC TTT AAT TTA AGT TCT ATC CCG ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 15  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 20  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 25  
 895 910 925 940  
 OCT GCA ACA GGC AAC TCG AAA TTC CCG GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 30  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCG GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 35  
 1000 1015 1030 1045  
 CCG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 40  
 1060 1075 1090 1105  
 TTG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 45  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 50  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 55  
 1225 1240 1255  
 AAA CAG CCG AAA CCG CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 60  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GGC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 65  
 1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 70  
 1390 1405 1420  
 AAT CAT GGC ATT GTT CAG ACG TTG CTC AAC TCT GTT AAC TCT AAG ATT CCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435                      1450                      1465                      1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

1495                      1510                      1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

1540(396)              1553              1563              1573              1583              1593              1603  
 TGT CCG TAGTACAGCA AATTAAATA CATAATATA TATATATATA TATATTTTAG AAAAAAGAA  
 Cys Arg

AAAA,

wobei das Verfahren die nachfolgenden Schritte umfaßt:

(a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,

(b) Isolieren positiver Clone und

(c) Isolieren der DNA-Insertionen aus diesen Clonen.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Gen menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist.

3. Verfahren zur Herstellung eines Gens, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;

(b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder

(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.

5. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.

6. Verfahren zur Herstellung eines Rinder-BMP-2 codierenden Gens, umfassend die nachfolgende DNA-Sequenz:

(1)

5 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
G H D G K G H P L H R R E K R

10 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG  
Q A K H K Q R K R L K S S C K

15 AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC  
R H P L Y V D F S D V G W N D

20 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG  
W I V A P P G Y H A F Y C H G

25 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT  
E C P F P L A D H L N S T N H

30 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC  
A I V Q T L V N S V N S K I P

35 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG  
K A C C V P T E L S A I S M L

40 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC  
Y L D E N E K V V L K N Y Q D

45 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCAGAGCA AAATAAATA  
M V V E G C G C R

50 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA

GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- 55 (a) Absuchen einer Genbank mit einer markierten auf der Grundlage der Aminosäuresequenz eines Fragmentes von bBMP-2 entworfenen Sonde, wobei die Genbank aus Rinderleber-DNA oder cDNA konstruiert wurde,
- (b) Isolieren positiver Clone und



(c) Isolieren der DNA-Insertionen aus diesen Clonen.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß das Gen Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 aufweist.

8. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

(b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder

(c) Fragmente, allelische oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.

10. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.

11. Verfahren zur Herstellung eines menschlichen BMP-4 codierenden Genes, das die nachfolgende DNA-Sequenz umfaßt:

10	20	30	40	50
CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCCGGC
60	70	80	90	100
CCGGAAGCTA	GGTGAGTGTG	GCATCCGAGC	TGAGGGACGC	GAGCCTGAGA
110	120	130	140	150
CGCCGCTGCT	GCTCCGGCTG	AGTATCTAGC	TTGTCTCCCC	GATGGGATTC
160	170	180	190	200
CCGTCCAAGC	TATCTCGAGC	CTGCAGCGCC	ACAGTCCCCG	GCCCTCGCCC
210	220	230	240	250
AGGTTCACCTG	CAACCGTTCA	GAGGTCCCCA	GGAGCTGCTG	CTGGCGAGCC
260	270	280	290	300
CGCTACTGCA	GGGACCTATG	GAGCCATTCC	GTAGTGCCAT	CCCGAGCAAC
310	320	330	340	350
GCACTGCTGC	AGCTTCCCTG	AGCCTTTCCA	GCAAGTTTGT	TCAAGATTGG
360	370	380	390	400
CTGTCAAGAA	TCATGGACTG	TTATTATATG	CCTTGTTTTTC	TGTCAAGACA

(1)  
CC ATG ATT CCT  
MET Ile Pro

5

417 432 447 462  
GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala

10 477 492 507  
AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCG GAG ATT CAG  
Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln

15 522 537 552 567  
GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CCG GAC TTC  
Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe

20 582 597 612 627  
GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CCG CAG OCT AGC AAG  
Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys

25 642 657 672  
AGT GCG GTC ATT CCG GAC TAC ATG CGG GAT CTT TAC CCG CTT CAG TCT GGG GAG  
Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu

30 687 702 717 732  
GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG CCG GCG GCG  
Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

35 747 762 777  
AGC CCG GCG AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile

40 792 807 822 837  
CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile

45 852 867 882 897  
OCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CCG CTC TTC CCG GAG CAG GTG  
Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val

50 912 927 942  
GAC CAG GGC OCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val

55 957 972 987 1002  
ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp

1017 1032 1047  
ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC OCT  
Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro

1052 1077 1092 1107  
GGG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCG ATT GAG  
Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu

1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT CCG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser  
 5  
 1182 1197 1212  
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GGC CAG CTC CCG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
 10  
 1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC CCG GGC CAT GGC TTG ACC CGA CCG CCG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys  
 1287 1302 1317  
 CGT AGC OCT AAG CAT CAC TCA CAG CCG GGC AGG AAG AAG AAT AAG AAC TGC CCG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 15  
 1332 1347 1362 1377  
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
 20  
 1392 1407 1422 1437  
 GGC CCA CCA GGC TAC CAG GGC TTC TAC TGC CAT GGC GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu  
 25  
 1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GGC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser  
 30  
 1497 1512 1527 1542  
 GTC AAT TCC AGT ATC CCC AAA GGC TGT TGT GTG CCC ACT GAA CTG AGT GGC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 1557 1572 1587  
 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 35  
 1602 1617 (408) 1636 1646 1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CCG TGAGATCAGG CAGTCTTGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
 40  
 1666 1676 1686 1696 1706  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CACGTTCCCA  
 50  
 1716 1726 1736 1746 1756  
 TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA  
 1766 1776 1786 1796 1806  
 TTAAAAA AAAA AATGGAAAA ATCCCTAAAC ATTCACCTTG  
 55

1816 1826 1836 1846 1856  
 ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA  
 5  
 1866 1876 1886 1896 1906  
 TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG  
 10  
 1916 1926 1936 1946  
 AGTCATTATT TTAAAAAATA AAAAAAACT CTAGAGTCGA CGGAATTC

wobei das Verfahren die nachfolgenden Schritte umfaßt:

(a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,

15

(b) Isolieren positiver Clone und

(c) Isolieren der DNA-Insertionen aus diesen Clonen.

20 12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß das Gen menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.

13. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:

25

(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 11 unterscheidet;

(b) mit einer DNA-Sequenz nach Anspruch 11 oder vorstehendem Absatz (a) hybridisiert; oder

30

(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

35

14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.

15. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.

40 16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.

17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.

45 18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.

19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.

50 20. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 zeigt, umfassend die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 1 bis 10 hergestellt wurden, und

55

- Gewinnen des Proteins aus dem Kulturmedium.

21. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-4 zeigt, umfassend die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 11 bis 15 hergestellt wurden, und

5        - Gewinnen des Proteins aus dem Kulturmedium.

22. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 oder BMP-4 zeigt, umfassend die Schritte

- 10        - Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
- 10        - Isolieren des Proteins aus dem Kulturmedium.

23. Verfahren zur Herstellung eines Arzneimittels, dadurch gekennzeichnet, daß es ein Kombinieren der nach einem der Ansprüche 20 bis 22 hergestellten Proteine, einzeln oder in Kombination, mit einem pharmakologisch verträglichen Träger umfaßt.

15

24. Verfahren nach Anspruch 23, dadurch gekennzeichnet, daß das Arzneimittel ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.

20

25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.

26. Verwendung eines Proteins nach einem der Ansprüche 20 bis 22, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

25

#### Revendications

30

Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

35

40

45

50

55

10 20 30 40 50 60 70  
 GTCGACTCTA GAGTGTGTT CAGCCTTGG CTGGGGACTT CTGAACTTG CAGGGAGAAT AACTTGGCA  
 5  
 80 90 100 110 120 130 140  
 CCCCCTTTG CGOOGGTGCC TTITGCCCCAG CCGAGCCTGC TTGGCCATCT CCGAGCCCCA CCGCCCCCTCC  
 10  
 150 160 170 180 190 200 210  
 ACTOCTGGC CTTGCCCCGAC ACTGAGAGCG TTITCCCCGC GTGAAAAGAG AGACTGCGCG GCGGGCACCC  
 15  
 220 230 240 250 260 270 280  
 GCGAGAGGA GGAGGCAAG AAAAGCAAG GACATTGGT CATTGCGCA GGTCCCTTGA CCGAGTTT  
 20  
 290 300 310 320 330 340 350  
 TCCATGTGA CGCTCTTCA ATGAGCTGT CCGCGGTGC TTCTAGAGC GACTCGGTC TCCCAAGGT  
 25  
 (1) 370 385 400  
 CGACC ATG GTG GGC GGG ACC GGC TGT CTT CTA GCG TTG CTG CTT CCC CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 30  
 415 430 445  
 CTC CTG GGC GGC GCG GGT GGC CTC GTT CCG GAG CTG GGC CCG AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 35  
 450 475 490 505  
 GCG GCG TCG TCG GGC CCG CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 40  
 520 535 550 565  
 TTC GAG TTG CCG CTG CTC AGC ATG TTC GGC CTG AAA CAG AGA CCC ACC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 45  
 580 595 610  
 AGG GAC GGC GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CCG AGG CAC TCG GTT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 50  
 625 640 655 670  
 CAG CCG GGC TCA CCC GGC CCA GAC CAC CCG TTG GAG AGG GCA GGC AGC CCA GGC  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala  
 55  
 685 700 715  
 AAC ACT GTG CCG AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA AGC  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730 745 760 775  
 AGT GGG AAA ACA ACC GGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 5  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 10  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 15  
 895 910 925 940  
 OCT GCA ACA GOC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 20  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 25  
 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GGC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 30  
 1060 1075 1090 1105  
 TTG CAG CAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 35  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 40  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT OCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 45  
 1225 1240 1255  
 AAA CAG CGG AAA CGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 50  
 1270 1285 1300 1315  
 TTC AGT CAC GTG GGG TGG AAT CAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GCC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 55  
 1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TGC OCT TTT OCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 1390 1405 1420  
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT OCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys

1435                      1450                      1465                      1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
 5  
                     1495                      1510                      1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GTG GAG GGT TGT GGG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly  
 10  
 1540(396)      1553              1563              1573              1583              1593              1603  
 TGT CGC TAGTACGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA  
 Cys Arg  
 15  
 AAAA

2. Gène codant pour la BMP-2 humaine comportant la séquence d'acides aminés donnée à la revendication 1.
3. Gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN :
  - (a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence de codons du fait de la dégénérescence du code génétique ;
  - (b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou
  - (c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non.
4. Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADN génomique.
5. Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADNc.
6. Gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :



(1)

5 GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
G H D G K G H P L H R R E K R

10 CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG  
Q A K H K Q R K R L K S S C K

15 AGA CAC CCT TTA TAT GTG GAC TTC AGT GTG GGG TGG AAT GAC  
R H P L Y V D F S D V G W N D

20 TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG  
W I V A P P G Y H A F Y C H G

25 GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT  
E C P F P L A D H L N S T N H

30 GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC  
A I V Q T L V N S V N S K I P

35 AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG  
K A C C V P T E L S A I S M L

40 TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC  
Y L D E N E K V V L K N Y Q D

45 ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA  
M V V E G C G C R

50 TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

AAGAAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA

GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT

7. Gène codant pour la BMP-2 bovine contenant la séquence d'acides aminés de la revendication 6.

8. Gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :

(a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

(b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe (a) ci-dessus ; ou  
 (c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication 7, que ces variations résultent de changements dans la séquence peptidique ou non.

5 9. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADN génomique.

10. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADNc.

11. Gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :

10  
 10 20 30 40 50 60 70  
 CTCTAGAGGG CAGAGGAGCA GCGAGGGAGG GAAGGAGGCG GGAGGCGGGC CCGGAGGCTA GGTGAGTGTG

15  
 80 90 100 110 120 130 140  
 GCATCGAGGC TGAAGGAGGC GAGCGTGAGA CGCGCTGCTT GCTCGGGCTG AGTATCTGAG TTGTCTGCCC

20  
 150 160 170 180 190 200 210  
 GATGGGATTC CCGTCGAGC TATCTGAGC CTGCAGGCGC ACAGTCCCGG GCGCTGCGCC AGGTTGACTG

25  
 220 230 240 250 260 270 280  
 CAACCGTTCA GAGGTCCCA GAGCTGCTG CTGGGAGAGC CGCTACTGCA GGGACCTATG GAGCCATTCC

30  
 290 300 310 320 330 340 350  
 GATGTCGAT CCGGAGCAAC GCACTGCTGC AGCTTCCCTG AGCCTTTCCA GCAAGTTTGT TCAAGATTGG

360 370 380 390 400 (1)  
 CTGTCAAGAA TCATGGACTG TTATATATATG CCGTGTCTTC TGTCAAGACA CC ATG ATT CCT  
 MET Ile Pro

417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 5  
 477 492 507  
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 10  
 522 537 552 567  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 15  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CCG CAG OCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 20  
 642 657 672  
 AGT GCC GTC ATT CCG GAC TAC ATG CCG GAT CTT TAC CCG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 25  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT CCT GAG CGC CCG GCC  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala  
 30  
 747 762 777  
 AGC CCG GCC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
 35  
 792 807 822 837  
 CCA GGG ACC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
 40  
 852 867 882 897  
 CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CCG CTC TTC CCG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
 45  
 912 927 942  
 GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
 50  
 957 972 987 1002  
 ATG AAG CCC CCA GCA GAA GTG GTG OCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
 55  
 1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CCG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
 1052 1077 1092 1107  
 GCG GTC CTT CGC TGG ACC CCG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
 1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT CCG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser

1162 1197 1212  
 CGA TCG TTA OCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
 5  
 1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG AOC CGA CGC CGG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys  
 10  
 1287 1302 1317  
 CGT AGC OCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 1332 1347 1362 1377  
 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
 15  
 1392 1407 1422 1437  
 GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Glv Trv Gln Ala Phe Trv Cvs His Glv Aso Cvs Pro Phe Pro Leu  
 20  
 1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser  
 25  
 1497 1512 1527 1542  
 GTC AAT TCC AGT ATC CCC AAA GCC TGT TGT GTG CCC ACT GAA CTG AGT GCC ATC  
 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
 1557 1572 1587  
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
 30  
 1602 1617 (408) 1636 1646 1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
 35  
 1666 1676 1686 1696 1706 1716 1726  
 ATATACACAC CACACACACA CACCACATAC ACCACACACA CAGCTTCCCA TOCACTCACC CAGACACTAC  
 40  
 1736 1746 1756 1766 1776 1786 1796  
 ACAGACTGCT TOCTEATAGC TGGACTTTTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC  
 45  
 1806 1816 1826 1836 1846 1856 1866  
 ATTCACTTIG AACTTETTTA TGACTTTACG TGCAATGTG TTGACCTAT TGACTATTA TTTTGACAA  
 1876 1886 1896 1906 1916 1926 1936  
 AATATTTAT AACTAGTAT TAAAGAAAA AATAAATG AGTCATTATT TTAATAAAAA AAAAAAAT  
 50  
 1946  
 CTAGAGTGA CGGAATC  
 55

12. Gène codant pour la BMP-4 humaine comportant la séquence d'acides aminés donnée à la revendication 11.

13. Gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :

- (a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique ;  
 (b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe (a) ci-dessus ; ou  
 (c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non.
14. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADN génomique.
15. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADNc.
16. Vecteur contenant le gène ou la séquence d'ADN suivant l'une quelconque des revendications 1 à 15, en association active avec une séquence de contrôle d'expression.
17. Cellule transformée avec un vecteur de la revendication 16.
18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.
19. Cellule suivant la revendication 18, qui est une cellule CHO.
20. Protéine montrant des propriétés de la BMP-2, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10.
21. Protéine montrant des propriétés de la BMP-2, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10, et de récupération de ladite protéine du milieu de culture précité.
22. Protéine montrant des propriétés de la BMP-4, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15.
23. Protéine montrant des propriétés de la BMP-4, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15, et de récupération de ladite protéine du milieu de culture précité.
24. Procédé de production de la protéine suivant l'une ou l'autre des revendications 21 et 23, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.
25. Composition pharmaceutique comprenant les protéines de l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, et un véhicule pharmaceutiquement acceptable.
26. Composition pharmaceutique suivant la revendication 25, comprenant de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et formant une structure pour induire une formation osseuse ou cartilagineuse.
27. Composition pharmaceutique suivant la revendication 26, dans laquelle ladite matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.
28. Utilisation d'une protéine suivant l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

#### Revendications pour l'Etat contractant suivant : AT

1. Procédé de préparation d'un gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

10 20 30 40 50 60 70  
 GTGACTCTA GAGTGTGTGT CAGCACTTGG CTGGGGACTT CTTGAACTTG CAGGGAGAAT AACTTGGCA  
 5  
 80 90 100 110 120 130 140  
 CCGACTTTTG CGCGGGTGCC TTGCGCCAG CCGAGCGTGC TTGGCATCT CCGAGCCCCA CCGCCCCCTC  
 10  
 150 160 170 180 190 200 210  
 ACTGCTGGC CTGCGCGAC ACTGAGACGC TGTTCOCAGC GTGAAAAGAG AGACTGGGCG GCGGCGACCC  
 15  
 220 230 240 250 260 270 280  
 GCGAGAAGGA GGAGGCAAAG AAAAGGAACG GACATTGGGT CCTTGCGCCA GGTCTTTTGA CCAGAGTTTT  
 20  
 290 300 310 320 330 340 350  
 TCCATGTGGA CGCTCTTTCA ATGACGTGT CCGCGGTGC TTCTTAGAGC GACTGGGGTC TCTAAAGGT  
 25  
 (1) 370 385 400  
 CGACC ATG GTG GCG GGG ACC CGC TGT CTT CTA GCG TTG CTG CTT CCG CAG GTC  
 MET Val Ala Gly Thr Arg Cys Leu Leu Ala Leu Leu Leu Pro Gln Val  
 30  
 415 430 445  
 CTC CTG GCG GCG GCG GGT GCG CTC GTT CCG GAG CTG GCG CGC AGG AAG TTC GCG  
 Leu Leu Gly Gly Ala Ala Gly Leu Val Pro Glu Leu Gly Arg Arg Lys Phe Ala  
 35  
 460 475 490 505  
 GCG GCG TCG TCG GCG CGC CCC TCA TCC CAG CCC TCT GAC GAG GTC CTG AGC GAG  
 Ala Ala Ser Ser Gly Arg Pro Ser Ser Gln Pro Ser Asp Glu Val Leu Ser Glu  
 40  
 520 535 550 565  
 TTC GAG TTG CGG CTG CTC AGC ATG TTC GCG CTG AAA CAG AGA CCC ACC CCC AGC  
 Phe Glu Leu Arg Leu Leu Ser MET Phe Gly Leu Lys Gln Arg Pro Thr Pro Ser  
 45  
 580 595 610  
 AGG GAC GCG GTG GTG CCC CCC TAC ATG CTA GAC CTG TAT CGC AGG CAC TCG GGT  
 Arg Asp Ala Val Val Pro Pro Tyr MET Leu Asp Leu Tyr Arg Arg His Ser Gly  
 50  
 625 640 655 670  
 CAG CCG GCG TCA CCC GCG CCA GAC CAC CGG TTG GAG AGG GCA GCG AGC CGA GCG  
 Gln Pro Gly Ser Pro Ala Pro Asp His Arg Leu Glu Arg Ala Ala Ser Arg Ala  
 55  
 685 700 715  
 AAC ACT GTG CGC AGC TTC CAC CAT GAA GAA TCT TTG GAA GAA CTA CCA GAA ACG  
 Asn Thr Val Arg Ser Phe His His Glu Glu Ser Leu Glu Glu Leu Pro Glu Thr

730 745 760 775  
 AGT GGG AAA ACA ACC CGG AGA TTC TTC TTT AAT TTA AGT TCT ATC CCC ACG GAG  
 Ser Gly Lys Thr Thr Arg Arg Phe Phe Phe Asn Leu Ser Ser Ile Pro Thr Glu  
 5  
 790 805 820 835  
 GAG TTT ATC ACC TCA GCA GAG CTT CAG GTT TTC CGA GAA CAG ATG CAA GAT GCT  
 Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Gln MET Gln Asp Ala  
 10  
 850 865 880  
 TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT ATT TAT GAA ATC ATA AAA  
 Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ile Tyr Glu Ile Ile Lys  
 895 910 925 940  
 OCT GCA ACA GGC AAC TCG AAA TTC CCC GTG ACC AGT CTT TTG GAC ACC AGG TTG  
 Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Leu Leu Asp Thr Arg Leu  
 15  
 955 970 985  
 GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT GTC ACC CCC GCT GTG ATG  
 Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp Val Thr Pro Ala Val MET  
 20  
 1000 1015 1030 1045  
 CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC GTG GTG GAA GTG GGC CAC  
 Arg Trp Thr Ala Gln Gly His Ala Asn His Gly Phe Val Val Glu Val Ala His  
 25  
 1060 1075 1090 1105  
 TTG CAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GTT AGG ATA AGC AGG TCT TTG  
 Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val Arg Ile Ser Arg Ser Leu  
 1120 1135 1150  
 CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA TTG CTA GTA ACT TTT GGC  
 His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro Leu Leu Val Thr Phe Gly  
 30  
 1165 1180 1195 1210  
 CAT GAT GGA AAA GGG CAT CCT CTC CAC AAA AGA GAA AAA CGT CAA GGC AAA CAC  
 His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu Lys Arg Gln Ala Lys His  
 35  
 1225 1240 1255  
 AAA CAG CGG AAA GGC CTT AAG TCC AGC TGT AAG AGA CAC CCT TTG TAC GTG GAC  
 Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg His Pro Leu Tyr Val Asp  
 40  
 1270 1285 1300 1315  
 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT CCC CCG GGG TAT CAC GGC  
 Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala  
 1330 1345 1360 1375  
 TTT TAC TGC CAC GGA GAA TCC CCT TTT CCT CTG GCT GAT CAT CTG AAC TCC ACT  
 Phe Tyr Cys His Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr  
 45  
 1390 1405 1420  
 AAT CAT GGC ATT GTT CAG ACG TTG GTC AAC TCT GTT AAC TCT AAG ATT CCT AAG  
 Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys  
 50  
 55

- 1435                      1450                      1465                      1480  
 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG  
 Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu  
 5
- 1495                      1510                      1525  
 AAT GAA AAG GTT GTA TTA AAG AAC TAT CAG GAC ATG GTT GIG GAG GGT TGT GGG  
 Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
- 10                      1540(396)                      1553                      1563                      1573                      1583                      1593                      1603  
 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTATG AAAAAAGAAA  
 Cys Arg
- 15                      AAAA ,
- dans lequel ledit procédé comprend les étapes suivantes
- 20                      a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de U-2 OS avec  
 un fragment de bBMP-2 marqué par hybridation,  
 b) l'isolement des clones positifs, et  
 c) l'isolement des inserts d'ADN de ces clones.
- 25                      2. Procédé suivant la revendication 1, dans lequel le gène code pour la BMP-2 humaine ayant la séquence d'acides  
 aminés donnée à la revendication 1.
- 30                      3. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et  
 comprenant une séquence d'ADN :  
 a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence des codons du fait de la dégé-  
 nérescence du code génétique ;  
 b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou  
 c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que  
 cette variation résulte de changements dans la séquence peptidique ou non,
- 35                      dans lequel le procédé susdit comprend des techniques standards de biologie moléculaire.
- 40                      4. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
5. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADNc.
- 45                      6. Procédé de préparation d'un gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :
- 50
- 55



(1) 15 30 45  
GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG  
G H D G K G H P L H R R E K R

5

60 75 90  
CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG  
Q A K H K Q R K R L K S S C K

10

105 120 135  
AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC  
R H P L Y V D F S D V G W N D

15

150 165 180  
TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG  
W I V A P P G Y H A F Y C H G

20

195 210 225  
GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT  
E C P F P L A D H L N S T N H

25

240 255 270  
GEC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC  
A I V Q T L V N S V N S K I P

30

385 300 315  
AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG  
K A C C V P T E L S A I S M L

35

330 345 360  
TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC  
Y L D E N E K V V L K N Y Q D

375 (129) 397 407  
ATG GTT GTC GAG GGT TGT GGG TGT CGT TAGCACAGCA AAATAAAATA  
M V V E G C G C R

40 417 427 437 447 457  
TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC

467 477 487 497 507  
ACTTTAATAT TTCCCAATGA AGACTTTATT TATGGAATGG AATGGAGAAA

45 517 527 537 547 557  
AAGAAAAACA CAGCTATTTT GAAACTATA TTTATATCTA CCGAAAAGAA

567 577 587  
GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,

50

dans lequel ledit procédé comprend les étapes suivantes :

- a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de foie bovin avec une sonde marquée conçue sur la base de la séquence d'acides aminés d'un fragment de bBMP-2,
- b) l'isolement des clones positifs, et
- c) l'isolement des inserts d'ADN de ces clones.

7. Procédé suivant la revendication 6, dans lequel le gène code pour de la BMP-2 bovine ayant la séquence d'acides

aminés de la revendication 6.

8. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :

5

a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe a) ci-dessus ; ou

10 c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication 7, que ces variations résultent de changements dans la séquence peptidique ou non,

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

9. Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADN génomique.

15

10. Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADNc.

11. Procédé de préparation d'un gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :

20

25

30

35

40

45

50

55

10 20 30 40 50 60 70  
 CTCCTAGAGGG CAGAGGAGGA GGGAGGGAGG GAAGGAGGCG GGAGGCGGCG CCGGAGGCTA GGTGAGTGTG  
 5  
 80 90 100 110 120 130 140  
 GCATGCGAGC TGAGGGAGCG GAGGCTGAGA CGGCGTGTCT GCTGCGGCTG AGTATCTAGC TTGTCTCCCG  
 10  
 150 160 170 180 190 200 210  
 GATGGGATTC CCGTCCAGC TATCTGAGC CTGCAGGCG ACAGTCCCG GCGCTGCGC AGGTTCACCTG  
 15  
 220 230 240 250 260 270 280  
 CAACGGTTCA GAGGTCCCA GGAGCTGCTG CTGGCGAGCG CGCTACTGCA GGGAGCTTGT GAGGATTC  
 20  
 290 300 310 320 330 340 350  
 GTAGTGCCAT CCGGCGAAC GCAGTGTGCG AGCTTCCCTG AGCGTTTCCA GCAAGTTTGT TCAAGATTGG  
 25  
 360 370 380 390 400 (1)  
 CTGTCAAGAA TCATGGACTG TTATTATATG CATTGTTTTC TGTCAAGACA CC ATG ATT OCT  
 MET Ile Pro...  
 30  
 417 432 447 462  
 GGT AAC CGA ATG CTG ATG GTC GTT TTA TTA TGC CAA GTC CTG CTA GGA GGC GCG  
 Gly Asn Arg MET Leu MET Val Val Leu Leu Cys Gln Val Leu Leu Gly Gly Ala  
 35  
 477 492 507  
 AGC CAT GCT AGT TTG ATA OCT GAG ACG GGG AAG AAA AAA GTC GCC GAG ATT CAG  
 Ser His Ala Ser Leu Ile Pro Glu Thr Gly Lys Lys Lys Val Ala Glu Ile Gln  
 40  
 522 537 552 557  
 GGC CAC GCG GGA GGA CGC CGC TCA GGG CAG AGC CAT GAG CTC CTG CGG GAC TTC  
 Gly His Ala Gly Gly Arg Arg Ser Gly Gln Ser His Glu Leu Leu Arg Asp Phe  
 45  
 582 597 612 627  
 GAG GCG ACA CTT CTG CAG ATG TTT GGG CTG CGC CGC CGC CGG CAG OCT AGC AAG  
 Glu Ala Thr Leu Leu Gln MET Phe Gly Leu Arg Arg Arg Pro Gln Pro Ser Lys  
 50  
 642 657 672  
 AGT GCC GTC ATT CGG GAC TAC ATG CGG GAT CTT TAC CGG CTT CAG TCT GGG GAG  
 Ser Ala Val Ile Pro Asp Tyr MET Arg Asp Leu Tyr Arg Leu Gln Ser Gly Glu  
 55  
 687 702 717 732  
 GAG GAG GAA GAG CAG ATC CAC AGC ACT GGT CTT GAG TAT OCT GAG CGC CGG GCC  
 Glu Glu Glu Glu Gln Ile His Ser Thr Gly Leu Glu Tyr Pro Glu Arg Pro Ala

747 762 777  
 AGC GGG GGC AAC ACC GTG AGG AGC TTC CAC CAC GAA GAA CAT CTG GAG AAC ATC  
 Ser Arg Ala Asn Thr Val Arg Ser Phe His His Glu Glu His Leu Glu Asn Ile  
 5  
 792 807 822 837  
 CCA GGG AOC AGT GAA AAC TCT GCT TTT CGT TTC CTC TTT AAC CTC AGC AGC ATC  
 Pro Gly Thr Ser Glu Asn Ser Ala Phe Arg Phe Leu Phe Asn Leu Ser Ser Ile  
 10  
 852 867 882 897  
 CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CCG GAG CAG GTG  
 Pro Glu Asn Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu Gln Val  
 15  
 912 927 942  
 GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG GTT  
 Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Asn Ile Tyr Glu Val  
 20  
 957 972 987 1002  
 ATG AAG CCG CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTG GAC  
 Met Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg Leu Leu Asp  
 25  
 1017 1032 1047  
 ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT GTG AGC CCT  
 Thr Arg Leu Val His His Asn Val Thr Arg Trp Glu Thr Phe Asp Val Ser Pro  
 30  
 1062 1077 1092 1107  
 GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG CTA GCC ATT GAG  
 Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Asn Tyr Gly Leu Ala Ile Glu  
 35  
 1122 1137 1152 1167  
 GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC CAG GGC CAG CAT GTC AGG ATT AGC  
 Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser  
 40  
 1182 1197 1212  
 CGA TGG TTA CCT CAA GGG AGT GGG AAT TGG GGC CAG CTC CCG CCC CTC CTG GTC  
 Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val  
 45  
 1227 1242 1257 1272  
 ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC CCG AGG GCC AAG  
 Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Ala Lys  
 50  
 1287 1302 1317  
 CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG  
 Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg  
 55  
 1332 1347 1362 1377  
 CGC CAC TGG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG  
 Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val  
 60  
 1392 1407 1422 1437  
 GCC CCA CCA GGC TAC CAG GGC TTC TAC TGC CTT GGG GAC TGC CCC TTT CCA CTG  
 Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Glv Asp Cys Pro Phe Pro Leu  
 65  
 1452 1467 1482  
 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT  
 Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser

1497                      1512                      1527                      1542  
 GTC AAT TOC AGT ATC CCC AAA GOC TGT TGT GTG CCC ACT GAA CTG AGT GOC ATC  
 5 Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile  
  
                     1557                      1572                      1587  
 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG  
 10 Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  
  
 1602                      1617                      (408)                      1636                      1646                      1656  
 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCTTGA GGATAGACAG  
 MET Val Val Glu Gly Cys Gly Cys Arg  
  
 1666                      1676                      1686                      1696                      1706                      1716                      1726  
 15 ATATACACAC CACACACCA CACACATAC ACCACACCA CAAGTTCCCA TOCACTCAOC CACACACTAC  
  
                     1736                      1746                      1756                      1766                      1776                      1786                      1796  
 20 ACAGACTGCT TCTTATATGC TGGACTTTTA TTTAAAAAA AAAAAAAAAA AATGAAAAA ATCTCTAAC  
  
                     1806                      1816                      1826                      1836                      1846                      1856                      1866  
 25 ATTCACTTIG ACCTTATTA TGACTTTACG TGCAATGTT TTGACCATAT TGATCATATA TTTTGACAAA :  
  
                     1876                      1886                      1896                      1906                      1916                      1926                      1936  
 ATATATTAT AACTAGTAT TAAAAGAAAA AATAAATG AGTCATTAT TAAAAAAA AAAAAAACT  
  
 1946  
 30 CTAGAGTGA CGGAATTC ,

dans lequel le procédé précité comprend les étapes suivantes :

- 35 a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant d'U-2 OS avec un fragment bBMP-2 marqué par hybridation,  
 b) l'isolement des clones positifs, et  
 c) l'isolement des inserts d'ADN de ces clones.
- 40 12. Procédé suivant la revendication 11, dans lequel le gène code pour la BMP-4 humaine ayant la séquence d'acides aminés donnée à la revendication 11.
- 45 13. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :
- 50 a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique ;  
 b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe a) ci-dessus ; ou  
 c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non,

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

- 55 14. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
15. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADNc.
16. Vecteur contenant le gène ou la séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 15, en

association active avec une séquence de contrôle d'expression.

17. Cellule transformée avec un vecteur de la revendication 16.

5 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.

19. Cellule suivant la revendication 18, qui est une cellule CHO.

10 20. Procédé de préparation d'une protéine montrant des propriétés de la BMP-2, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 10 et de récupération de ladite protéine du milieu de culture précité.

15 21. Procédé de préparation d'une protéine montrant des propriétés de la BMP-4, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 11 à 15 et de récupération de ladite protéine du milieu de culture précité.

20 22. Procédé de production d'une protéine montrant des propriétés de la BMP-2 ou BMP-4, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.

25 23. Procédé de préparation d'une composition pharmaceutique comprenant la combinaison des protéines préparées suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison avec un véhicule pharmaceutiquement acceptable.

30 24. Procédé suivant la revendication 23, dans lequel la composition pharmaceutique susdite comprend de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et constituer une structure pour induire une formation osseuse ou cartilagineuse.

25 25. Procédé suivant la revendication 24, dans lequel la matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.

35 26. Utilisation d'une protéine préparée suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

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